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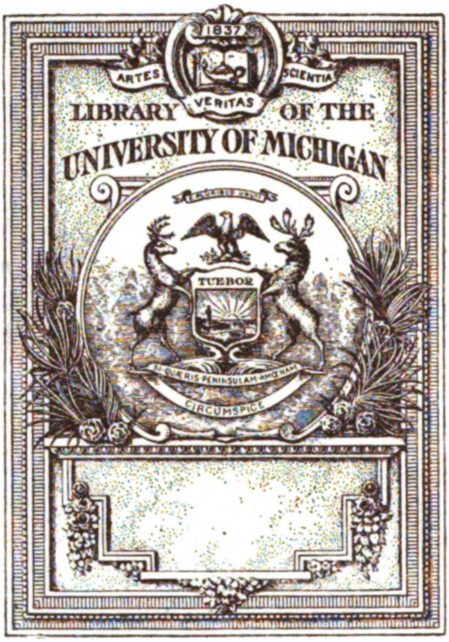

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# CONTRIBUTIONS
FROM THE
ZOOLOGICAL LABORATORY
FOR THE YEARS
1915-17

VOLUME XX
THIRTY SEPARATA

PHILADELPHIA, PA.
1918

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*2. **Calvert, Philip P., and Lyon, Mary B.** Miscellaneous Notes on Odonata. With Comments on the Dimorphism of the Females of Ischnura Verticalis. (Entomological News, Vol. XXVI, February, 1915.)

3. **Calvert, Philip P.** Studies on Costa Rican Odonata. VI. The Waterfall-Dwellers: The Transformation, External Features and Attached Diatoms of Thaumatoneura Larva. (Entomological News, Vol. XXVI, July, 1915.)

4. **Calvert, Philip P.** Studies on Costa Rican Odonata. VII. The Waterfall-Dwellers: The Internal Organs of Thaumatoneura Larva and the Respiration and Rectal Tracheation of Zygopterous Larvæ in General. (Entomological News, Vol. XXVI, November, 1915.)

5. **Calvert, Philip P.** Studies on Costa Rican Odonata. VII (Continued). The Waterfall-Dwellers; The Internal Organs of Thaumatoneura Larva and the Respiration and Rectal Tracheation of Zygopterous Larvæ in General. (Entomological News, Vol. XXVI, December. 1915.)

6. **Calvert, Philip P.** Studies on Costa Rican Odonata. VIII. A New Genus Allied to Cora. (Entomological News, Vol. XXVIII, June, 1917.)

7. **Carothers, E. Eleanor.** The Segregation and Recombination of Homologous Chromosomes as found in Two Genera of Acrididæ (Orthoptera). (Journal of Morphology, Vol. 28, No. 2, March, 1917.)

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11. Colton, Harold Sellers. On Some Varieties of Thais Lapillus in the Mount Desert Region. A Study of Individual Ecology. (Proceedings of the Academy of Natural Science of Philadelphia, June, 1916.)

12. Colton, Harold Sellers. Is the House of Tcuhu the Minoan Labyrinth? (Science, N. S., Vol. XLV, No. 1174, June, 1917.)

13. Hance, Robert T. A System for Recording Cytological Material, Slides and Locations on the Slides. (American Microscopical Society, Vol. XXXV, No. 1, January, 1916.)

14. Hance, Robert T. A Miniature Dark Room for Use with the Microscope. (American Microscopical Society, Vol. XXXV, No. 1, January, 1916.)

15. Hance, Robert T. Notes on Handling Protozoa in Pure Line Work. (American Microscopical Society, Vol. XXXV, No. 2, April, 1916.)

16. Hance, Robert T. Notes on Embedding in Paraffin. (American Microscopical Society, Vol. XXXV, No. 2, April, 1916.)

17. Hance, Robert T. A Simple Paraffin Ribbon Winder. (The Anatomical Record, Vol. 10, No. 8, June, 1915.)

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26. McClung, Clarence E. Cooperative Technique. (The Anatomical Record, Vol. 12, No. 3, April, 1917.)

27. McClung, Clarence E. The Multiple Chromosomes of Hesperotettix and Mermiria (Orthoptera). (Journal of Morphology, Vol. 29, No. 2, September, 1917.)

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STUDIES ON CELL DIVISION IN THE ALBINO RAT (MUS NORVEGICUS, VAR. ALBA)

II. EXPERIMENTS ON TECHNIQUE, WITH DESCRIPTION OF A METHOD FOR DEMONSTRATING THE CYTOLOGICAL DETAILS OF DIVIDING CELLS IN BRAIN AND TESTIS

EZRA ALLEN

The Philadelphia School of Pedagogy and the Zoological Laboratory of the University of Pennsylvania

ONE TEXT FIGURE AND TWO PLATES

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INTRODUCTION

This paper deals with a series of experiments on technique by which a method has been developed for demonstrating the details of mitosis in the central nervous system and testis of the albino rat without producing distortion of the other parts of the tissues. The work was begun because of difficulty experienced in positively identifying dividing cells while conducting experiments on influencing the rate of cell division in the cerebellum of the albino rat. In the external granule layer of a sick individual seven days old, I found a large number of peculiar cells which oftentimes resembled certain phases of dividing cells. The tissue had been fixed in Carnoy's fluid and treated according to the method previously used in my study determining the duration of cell division in the central nervous system of the albino rat (Allen '12). At Professor McClung's suggestion I attempted to secure a technique which would insure the cytological details desired.

King ('10), after a thorough and exhaustive set of experiments on various standard fixatives for brain tissue, showed that for general histological purposes Ohlmacher's fixing fluid followed by double imbedding with celloidin and paraffin gave the best results. This method, however, fails to prevent the chromosomes from fusing, and consequently was inadequate for the purpose in hand.

My experiments grew to a series of tests dealing with each step in the preparation of sections, finally resulting in a method which not only clearly differentiates dividing cells in all stages, but also prevents fusing, or 'clumping,' of the chromosomes, a distinct gain in the technique for mammalian tissue. Other workers in the Zoological Department of the University of Pennsylvania are experimenting on other tissues. The results of their work will also be published, so that this paper forms one of a series on the problems of technique.

THE SCOPE OF THE EXPERIMENTS

It is needless to record all the details of the work done or of the results obtained. The general plan and scope of the work are as follows: While a few experiments were made on a pure trial and error basis, with the hope that something desirable would result, most of them were carefully planned along the line of modifying approved methods. The success of each experiment was measured by two criteria: (1) the isolation and definition of the chromosomes and (2) the 'general fixation.' The term 'general fixation' covers the points of shrinkage or swelling of the whole mass, and the degree of distortion of one part or of one tissue. A good general fixation describes the condition of a preparation which has suffered only slight changes in total volume, either by shrinkage or swelling, and in which the individual parts maintain their normal relationship of volume and structure; that is, the connective tissues are not torn, the limiting membranes retain their size and smooth outlines, while the cells show no vacuoles about them nor cytolysis of any kind.

For a long time it looked as if excellence of general fixation would have to be sacrificed to distinctness of the chromosomes, so that I was particularly pleased when the best chromosome differentiation obtained was accompanied by the best general fixation. A comparison of figures 2, 9, 10, 13 and 15 with figures 4, 7, 12, 14 and 17 respectively will show the contrast between poor and good preparations according to the standards just described.

EXPERIMENTS ON FIXATIVES

The first line of investigation followed was that of modifying the proportions of constituent parts of Bouin's fluid, since this fixative had proved so excellent for general purposes. The proportions are given in table 1. The first set of experiments was with the first six modifications shown in table 1. One promising fluid appeared in modification no. 2. It failed, however, upon further trial to yield constant or fully satisfactory results, though it seems to be a better fixative for albino rat tissue than Bouin's combination.

After thorough trial of the picro-formol-acetic combinations as far as no. 14 in the table, the following fluids were used: Flemming's, Worcester's, Lavdowsky's, Carnoy's (often called van Gehuchten's), Ohlmacher's, King's potassium-bichromate-acetic-sublimate, and Tellyesniczky's. Urea was added to each in varying proportions for studying its effects; fixatives were made up in normal salt solution; different temperatures—0°C., room and 30 to 40°C.—and different fixation periods were tried with any

TABLE 1

Showing the various combinations of picric acid, formol, acetic acid, corrosive sublimate, chromic acid and urea employed. Fluids are measured in cubic centimeters; solids, in grams; the percentages are thus indicated

SOLUTION	PICRIC ACID; SATURATED AQUEOUS SOLUTION	FORMOL	GLACIAL ACETIC ACID	CORROSIVE SUBLIMATE SATURATED; AQUEOUS SOLUTION	SALT; CRYSTALS	UREA; CRYSTALS	CHROMIC ACID; CRYSTALS	WATER
Bouin[1]	75	25	5					
No. 1	75	15	5					
2	75	10	10					
3	75	15	10					
4	75	10	5					
5	75	5	5					
6	75	10	10	5				
7	50	10	10					25
8	90	5	5		0.9	0.5		
9	75	10	10		0.9	1.5		
10	75	15	10		0.9	1.5		
11	75	10	10			0.1		100
12	75	10	10			1.5		300
13	75	25	5			4.0		300
14	75	25	5		0.9	0.5		
15	75	25	5			2.0	1.5	
16	75	20	3			1.0	1.0	
17	75	5	1			1.0	0.5	
18	75	20	7			0.3	0.5	

[1] Lee ('13).

fluid which seemed hopeful on first use. Various strengths of formalin, with and without sugar, and different combinations of acetone and formalin were also tried. See table 2.

A second promising fluid resulted from the addition of a little dilute chrome-acetic mixture (one of the parts of Flemming's fluid) to the Carnoy fluid. The general fixation was excellent and the dividing cells in the brain showed better cytological detail than with anything else tried up to that time. This combination was therefore tested. The constituents were mixed in different proportions; and different temperatures, periods of fixation and quantities of urea tried out. Chromic acid yielded better and better results upon the chromosomes as its percentage was increased, but the mitotic figures were still not satisfac-

TABLE 2

Showing some of the various combinations of fluids used based upon either formol, acetone or Carnoy. The measurements are the same as in table 1

SOLUTION	FORMOL (NEUTRAL)	ACETONE	ALCOHOL (100 PER CENT)	CHLOROFORM	GLACIAL ACETIC ACID	CHROMIC ACID; CRYSTALS	CORROSIVE SUBLIMATE; CRYSTALS	UREA; CRYSTALS	SUGAR; CRYSTALS	WATER
No. 1 McClendon	10								2.0	88
2	10							1	1.0	90
3	10								0.5	90
4	1							1	1.0	98
5	2							1	1.0	98
6	5							1	1.0	94
7	2		48					1		50
8	1	90						1		10
9	10	50						1	1.0	40
10	5	50						1	1.0	40
11	10		50					1		45
12	5		50					1	1.0	45
Carnoy			60	30	10					
13			60	30	5	1.0				
14			98			5.0				2
15			60	30	10	1.5		2		
16	5		60	30		1.5		2		2
17			60	30		1.5		2		2
Ohlmacher			80	15	5		Saturation			

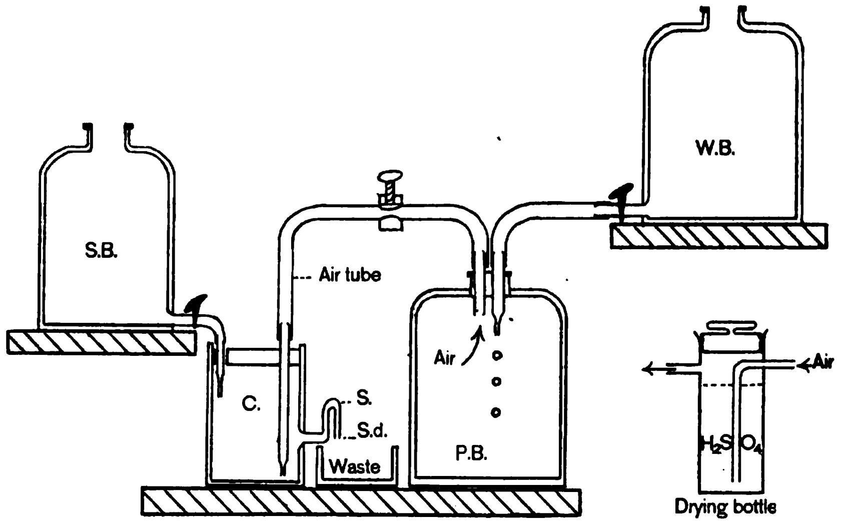
tory for accurate work when the concentration had reached a point in which the rest of the tissues were torn and distorted worse than the results of Flemming's fluid shown in figure 13. Some of the combinations used are shown in table 2, nos. 13 to 17. Formol and acetic acid both swell tissues. For this reason formol was substituted for the acetic acid in several trials, only one of which is shown in the table (no. 16, table 2).

Finally one half of a seven day brain was put into the combination of chromic acid and Carnoy's fluid which had given the best results, and the other half in some Bouin's fluid to which I added at the same time some chromic acid and urea. The half placed in the latter fluid showed all the good points of general fixation gotten by Bouin's fluid with the addition of well differentiated chromosomes, while the other half was useless in comparison. Further trials of the new fixative (no. 15, in table 1) yielded practically constant results and later when applied to the testis gave the material from which figures 12, 14 and 17 were made. The exact proportions used appear in table 1, no. 15. This fixative will henceforth in this paper be spoken of as the picro-formol chrome-acetic in urea fixative, or for convenience 'B-15.'

DEHYDRATION

The well recognized necessity of a very slow change from a fluid of one density and osmotic pressure to another which differs in these respects, in order that cytolysis may be avoided, led to a consideration of how this change might be easily and effectively accomplished. Professor McClung while working on grasshopper testis found that if the higher grades of alcohol were added drop by drop better results were obtained than by transferring the tissue from water to 35 per cent, 50 per cent, 60 per cent alcohol, etc. To accomplish this purpose he used the principle of capillarity in a cotton cord or a fine glass siphon. This method, however, when applied to more than two or three cubic centimeters of fluid did not insure mixing, the lighter liquid remaining on top of the heavier. Even when the lighter liquid is introduced through a tube to the bottom of the heavier, it rises immediately and accumulates on top of the heavier in an independent layer and remains there for days, as is readily shown by introducing colored alcohol in the manner described. I have found that a flow of air gently and rapidly mixes the fluids of different density. The current may be obtained from a pressure bottle when compressed air is not available in the laboratory. The apparatus devised for this purpose is shown in text-figure 1. It consists of two bottles of equal volume, one for water and the other for air pressures, marked *W.B.* and *PB.* One of these is of the aspirator type. The remainder of the apparatus is a supply bottle for storing the alcohol or other replacing fluid, *S.B.*, also fitted with bottom stopcock; a container for the tissue, *C*, fitted with a siphon opening from the side, *S*; a jar for receiving waste; glass and rubber tubing for connections as shown; corks and screw clamps or stopcocks of small bore, as indicated in the figure.

The size of the water and pressure bottles should be two or more liters, in order that little attention need be given the supply of air. A variety of sizes of the container is desirable, since a small amount of tissue needs only a small amount of fluid. For two or three pieces of 0.5 cc. volume a vessel of 30 cc. total capacity is ample. Shell vials or shallow stender dishes are very suitable. One should avoid narrow, tall containers as handling the tissue in them is difficult. The supply bottle for alcohol may be large or small, depending upon the quantity of fluid needed. Where large quantities of tissue are being dehydrated or changed, several supply bottles will be convenient, one for each kind of fluid that is in use.



If aspirator bottles are not available, ordinary bottles fitted with siphons through their corks may be substituted.

The connections should be made as shown in the diagram. The cork for *P.B.* fits tightly; that for *C* is loose-fitting, or else the hole for one of the tubes is slightly larger than the tube, to allow for escaping air. The cork for *S.B.* must also fit loosely.

To operate: after seeing that all connections are adjusted, fill the water bottle *W.B.* and open its stopcock slightly until the water begins to drop into the pressure bottle. As soon as the air pressure in this bottle overcomes the pressure of the fluid in *C*, bubbles will emerge from the mouth of the air tube and pass to the top of the fluid. A steady stream of air instead of bubbles is better, and may be produced by drawing out the end of the air tube into almost a capillary and clamping its rubber tube almost shut, thus forcing the air to accumulate under pressure in *P.B.*

Next start the alcohol dropping at the desired rate. The air and alcohol will continue to flow until their supply is exhausted in each case or until stopped by the operator. The siphon will remove the excess of fluid, so that the concentration of the alcohol is steading rising in *C*. The waste jar must be large enough to accommodate the excess fluid.

A two-liter bottle for *W.B.* if filled will run all night at the rate of a drop a second. When the water has run over into the pressure bottle it is poured back into the water bottle and thus used repeatedly.

When the apparatus is used for higher alcohols or for oils, the air should be dried by passing it through a tube containing calcium chloride or through sulphuric acid. The latter method is easily accomplished by a gas washing bottle, shown at one side in the diagram.

As a concrete illustration, suppose that the tissue is to be raised from water to 75 per cent alcohol. It is placed in the container *C* and covered with the fixing fluid or with whatever fluid it may be in at the time. The air and alcohol are set flowing as previously described. Sometimes a difficulty appears in connection with the stopcocks or pressure clamps. Stoppage will occur in the rubber tubing so clamped after the clamp has been on for some days. Consequently the clamp must be moved occasionally. The stopcocks need turning wide open occasionally and then readjusted to the desired rate of flow.

The rate of dropping is affected by the pressure of the water above the stopcock in the water bottle, this pressure varying directly with the quantity of water. A little experimenting will usually suffice to regulate the flow satisfactorily.

If the tissue is very delicate and is seen to be moving about in the container, the rate of air admission may be slowed or a glass slide interposed between the air and the tissue. This should fit loosely enough to allow of free interchange of liquids about its edges but to prevent rapid currents at the same time. Another excellent scheme is to put the tissue into a smaller bottle, the sides of which have been perforated with numerous holes. This receptacle prevents the air currents from forcibly disturbing the tissue while at the same time it permits very free circulation of the fluids.

The siphon may be adjusted to discharge constantly, once it has started, or intermittently. If its discharge is at the same level as the point where it leaves the container (as shown in the cut), the flow will be constant. If the descending arm is longer than the ascending arm, thus lowering the discharge point, the flow will be intermittent.

By measuring the amount of water in the container at the beginning, one can readily calculate how much alcohol of a given strength will be required to bring the fluid in the container up to 70 per cent, or any other percentage desired. This quantity can be put into the supply bottle *S.B.*

The percentage of alcohol to be used at first varies somewhat with conditions. If the tissue is small compared with the volume of fluid in the container, 95 per cent may be used. On the other hand, if the quantity of fluid is small compared with the tissue it is better to start with 50 per cent, or 70 per cent and continue this until the tissue is in 50 per cent when 95 per cent may be run in. The new fluid may be introduced with constantly increasing speed as its concentration rises in the container.

The same apparatus is very convenient for washing out picric acid or corrosive sublimate, as the current of air hastens the process considerably, since the agitation constantly removes the layer of fluid which exudes from the tissue, and the interchange is thus not dependent solely upon diffusion of fluids. If desired, alcohol of the same strength may be kept running into the container from the supply bottle; or the fluid in the container may be changed at intervals.

For passing to the higher alcohols and clearing oils, the same apparatus is employed in the same way as for bringing the tissue up to 70 per cent.

THE USE OF ANILIN IN PLACE OF THE HIGHER ALCOHOLS

While carrying on these experiments at Woods Hole, Dr. E. E. Just called my attention to Suchannek's ('90) use of distilled anilin oil as a substitute for the higher alcohols, and spoke of his own success in its use on heteronereis eggs. I tried it after 75 per cent alcohol. It produced no appreciable change in the volume of the tissue even if left in it for twenty-four hours, and I have since employed it entirely except after a Flemming fixation. Tissue so fixed is turned very black by it, even though previously very thoroughly washed.

Anilin does not mix with paraffin but is readily followed by any clearing oil. I have had no difficulty with its use. On the other hand, it seems to improve the tissue for sectioning and staining. By one distillation (the distillation point is 184°C.) anilin becomes a clear, pale-yellowish fluid. This keeps well if stored in the dark. The Kahlbaum anilin comes a pale red and can be used without distillation, but may darken the tissue somewhat.

King ('10) found that double imbedding prevented shrinkage of the nerve cell body. She says:

> When a brain that has been fixed in Ohlmacher's solution is imbedded in paraffin after being cleared with chloroform or with any other substance commonly used for this purpose, there is invariably a shrinkage of the cell body, ... and a condensation or vacuolization of the cytoplasm. If, however, the brain is imbedded in celloidin-paraffin, ... there is no shrinkage evident anywhere in the cell.

referring to large cells in the cerebral cortex. I am confident that the use of anilin obviates this difficulty, and consequently avoids the shrinkage due to the higher alcohols essential to the use of celloidin, and furthermore gives perfectly flat sections. The photographs in figures 3 and 4 are typical of the appearances produced by my method of treatment. Neither large nor small cells show vacuolization of cytoplasm nor shrinkage of the nucleus.

CLEARING

Xylol, as is well known (Lee '13) shrinks tissues considerably. I experimented with chloroform, oils of cedar, cassia, wintergreen and bergamot. Each produced less shrinkage than xylol. The effects in each case were measured on the whole mass of tissue, on the smoothness of outline of the individual tubules of the testis, and on nerve cells. The least shrinkage occurred with bergamot; wintergreen is a close second. Cassia (cinnamic aldehyde) seemed excellent for the testis, but since this does not mix with paraffin it must be followed by a paraffin solvent. It has the advantage of mixing with 85 per cent alcohol freely, and it is therefore useful after Flemming to avoid the higher alcohols. The pure synthetic preparations of all the oils are better than the natural products. The following illustrations serve to show the extent of shrinkage during the clearing process.

	cc.
Volume of brain when put into xylol	0.65
Volume of brain 2½ hours later	0.60
Volume of brain 23 hours later	0.50

The loss in this case was 23 per cent. In some cases it was as high as 25 per cent.

The effects of bergamot oil were compared with those of a special cedar oil which has the merit of mixing with alcohol in any proportion. I passed the halves of the same cerebellum through these oils respectively, and then measured ten undeveloped Purkinje cells from each preparation, carefully selecting those cut in the same way. Drawings were made with the aid of the camera lucida. The mean diameter of the ten fixed in bergamot oil was 9 units; that of the ten fixed in the special cedar oil was 8.1 units, a loss of 10 per cent. The diameters of the latter were consistently smaller throughout.

Testis fixed in Flemming seems less susceptible to change in volume at this stage in the technique than when fixed in one of the picro-formol-acetic combinations, an indication that this fixative (Flemming) has brought the tissue more nearly to its limit of shrinkage.

DETAILED STATEMENT OF THE METHOD

Formula for the fixative. (B-15; table 1, no. 15)

	cc.
Picric acid, saturated aqueous solution	75
Formol	25
Glacial acetic acid	5

In this fluid dissolve at time of fixing 1.5 gram of chromic acid in crystals, and then 2 grams of urea in crystals.

Fixation. Heat the fixative to 37° or 38°C., place the tissue in it and hold at that temperature. Pieces of brain 0.5 cc. in volume fix in one hour; pieces of young testis of the same or less volume require a little longer. The adult testis should be given two to three hours, depending on the age of the animal.[1] The external membranes of the tubules and the connective tissues become, with age, progressively less easy to penetrate. Plenty of fixing fluid is used. It is agitated several times during the fixation. To secure quick penetration of the testis it is necessary to cut the outer covering (the tunica albuginea), and in addition snip the tubules apart with scissors.

A low temperature paraffin bath is convenient for keeping the fluid at the desired temperature.

Dehydration. The fluid and contained tissue are cooled to room temperature and slow, gradual dehydration begun with alcohol as described previously (p. 578). The rate of dropping should be about one drop per second unless the quantity of fluid is very small, when it should be less rapid.

After this fixative, it is desirable to bring the tissue up to 75 per cent within an hour (for pieces 0.5 cc. in volume) if the tissue is soft; harder tissues need a little more time.

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[1] The young mature testis is better for the study of spermatogenesis than the old.

Since the picric acid must be washed out, fresh 75 per cent alcohol to which a few drops of a saturated aqueous solution of lithium carbonate has been added is placed on the tissue in the supply bottle, and the flow of air started very slowly. By agitation and constant drainage, the time for washing out picric is lessened to one-third that required when the tissue simply soaks in the same solution, which is renewed from time to time. Very small pieces will wash in a few hours. Large pieces must be left until no yellow appears in the fluid.

It is desirable to keep the tissue in alcohol as short a time as possible. As noted in table 3, the shrinkage that occurs in getting the tissue into this grade of alcohol is considerably more than occurred in the fixative.

The anilin oil is started dropping slowly. It is heavier than alcohol so that a stronger current of air may be needed to insure quick mixing. The eye will readily detect the flow of air necessary. When nearly pure anilin is reached, the tissue is left in pure anilin until it is clear like amber.

Clearing. The oil follows by the same method. The tissue should be changed once after being placed in the pure oil, as it is quite essential that all of the anilin be removed, or poor infiltration by paraffin results.

Infiltration with paraffin. This is accomplished by warming the oil and tissue slightly and adding to it every ten minutes a few drops of melted paraffin, which is then thoroughly mixed with the oil by a pipette. This is continued until the mixture is 85 per cent or 90 per cent paraffin. It is then transferred to the melted paraffin. I use one melting point only—52° or 55°C.

If bergamot oil has been used, at least four changes of paraffin should be made, the tissue remaining in each a half hour, and in the fifth paraffin at least one hour. Testis requires longer. I cannot see that twelve to twenty hours in paraffin of 55°C. injures brain or testis.

Imbedding. This is done in the usual way.

RESULTS OF THE METHOD DEVELOPED

Cytological. As stated in the introduction, the object of the experiments was to obtain a method which would positively differentiate dividing cells in the brain of the albino rat. This object was attained. In addition, the same method has given the most satisfactory preparation of the testis yet obtained for the study of spermatogenesis, as simultaneously with the work on the brain tissue the testis was also tested. On this latter material cold Flemming's fluid (0°C.) to which was added 2 or 3 per cent urea gave some very admirable results, but of many testes fixed only two came out well, and these were good only in a few places, while the tubules that showed chromosomes in good condition for study were invariably badly torn, as is illustrated in figures 13 and 15. Figure 10 was made in order to show that this torn condition is general and not confined to one or two places. All stages of spermatogenesis are shown in the tubules of figure 10. It is evident that any conclusions relative to chromosomes drawn from tissues so badly distorted should be fully controlled by a preparation in which no such distortion is to be seen. It is believed that figure 14 illustrates such a preparation.

In the Flemming-fixed material, the early prophases seem more sharply defined, as if the chromatin were compressed into more of a dense thread than in the picro-formol-acetic preparations. It is likely that the latter fixation preserves them in a more nearly normal condition. When chromic acid and urea are added to the picro-formol-acetic mixture the metaphase chromosomes of both spermatocytes are held in some way from clumping, just as they are in the best Flemming preparations, while the prophase threads are not hardened down appreciably. The chromatin maintains its diffuse character between the chromomeres. It may be that on this account the study of these prophase chromosomes will be easier in Flemming-fixed material if one desires to differentiate a single chromosome in the large number possessed by rat and some other mammals, but for the study of the structure of the individual chromosomes, it would seem that the

B-15 preparation is decidedly preferable. I shall have more to say upon this subject in the paper bearing upon the spermatogenesis of the albino rat.

In this paper, however, I wish merely to show the possibilities of isolating chromosomes in the metaphases of spermatocytes by the addition of urea to Flemming's fluid and fixing at 0°C. Figure 15 is to be contrasted with figure 16, where the chromosomes are so clumped as to be hopelessly unresolvable with the best microscopic combinations available. Figure 17, prepared by the B-15 fixative, does not show its full worth in this figure. I had no slide of that preparation which showed several metaphase conditions in polar view, and was compelled consequently to use lateral views at this magnification, trusting that the higher magnification shown in figures 14 and 14 a would bear witness to the real excellence of the preparation. Furthermore only a few cells in figure 17 focussed at the same level, while many did in figure 15. The excellence of general fixation comes out clearly in both figures 14 and 17.

In this connection, attention is called to the apparent excellence of fixation in figure 11. This contrasts favorably with figure 10. Examination of figure 16, however, which is made from the same slide as figure 11, shows the clumped condition of the chromosomes and some spaces about the cells. Neither of these conditions is manifest in figure 17. The spaces near the center of this last figure are the normal lumen of the tubule.

Consider now the cytological details of the brain tissue as illustrated in the figures. The superiority of the general fixation shown in figures 3 and 4 over that shown in figure 2 is manifest. The spaces about the cells in figure 2 do not appear in tissue fixed by B-15 and subsequently treated by the method described in this paper. Tissue fixed in Carnoy's fluid, in formalin, in alcohol and in Ohlmacher's fluid show these spaces throughout. Figures 3 and 4 are typical of the preparations by the method which I have employed in connection with the picroformol-chrome-acetic-urea fixative. No such spaces appear about cells of any size. Large cells have been purposely chosen for the figures, as they would show such spaces most strikingly if present.

In these cells the characteristic fibrillation of the larger protoplasmic process appears distinctly. Fibers are also distinct between the cell bodies. The chromatin masses in the nucleus are more delicately treated than in the other fixations.

The equal action of B-15 throughout the tissue is illustrated in figures 6 and 7. The latter figure shows the outer part of the external granule layer, or where this layer lies on the outside of the cerebellum, while figure 6 shows the same layer deep within a sulcus, as at *I*, figure 1. The cells in each region seem alike. Those in figure 9, on the contrary, are markedly different from those in figure 8. Figure 9 shows the outer cells and figure 8 the deep-lying ones. The appearance in figure 9 is characteristic of Carnoy, alcohol and Ohlmacher preparations, which therefore prove very unsatisfactory fixatives if one desires to study this layer of cells throughout.

The demonstration of dividing cells in photographs was found very difficult for several reasons. One is that the chromosomes are very small and numerous, and that they do not form a flat equatorial plate. A photograph of any equatorial plate would therefore appear to show some fusing. In fact, if one wishes to be absolutely sure of the number of chromosomes, the cell must be slightly inclined to the plane of section, since in a strict polar view one or more of the chromosomes may be entirely hidden by another chromosome. Figure 5 shows a dividing cell in early anaphase, in which some of the chromosomes may be seen separately at this focus. In the preparation they are perfectly distinct from each other. In figure 6, one of the daughter nuclei of a dividing cell in late anaphase is seen (*D. c.* in lower part). This stage is the most critical one in the fusing of the chromosomes. In all other fixatives they mass into a solid body at this stage. In this figure, however, they will be seen as distinct, although too small to be isolated in the photograph. The dividing cell in the upper part of this figure is out of focus, but it can be seen that its edge is not as hard and smooth as the dividing cell in figure 8, which is typical of all other fixatives except B-15.

SHRINKAGE DUE TO FIXATION

Several brains were weighed to show the change in volume due to fixation. The data appear in table 3. The method of weighing is as follows: as soon as the brain is removed it is dipped for a few seconds into the fixative, then removed and drained on filter paper. It is then placed in a weighing bottle and the weight of brain and bottle determined. The brain is then removed and the bottle weighed before cleaning. The difference between the first and second weights is taken as the brain weight. If the brain is weighed as it comes from the animal, its condition is not the same after being in the fixing fluid, as some of the fluid will remain on its surface. This may be sufficient, even after draining, to counterbalance the loss of weight due to the action of the fixative.

TABLE 3

Showing loss in weight of different-aged brains in the fixing fluid which gave the best results, viz., picro-formol chrome-acetic urea. See table 1, no. 15

NUMBER OF RAT	SEX	AGE IN DAYS AFTER BIRTH	NUMBER OF HOURS IN FIXING FLUID	WEIGHT IN GRAMS BEFORE FIXATION	WEIGHT IN GRAMS AFTER FIXATION	LOSS OF WEIGHT IN FIXING FLUID	PERCENTAGE LOSS OF WEIGHT IN FIXING FLUID
133	♂	2	1	0.2860	0.2640	0.0220	7.6
119	♀	3	1	0.2992	0.2770	0.0221	7.4
117	♂	7	1	0.6605	0.6024	0.0581	8.8
118	♀	7	1	0.6799	0.6206	0.0593	8.7
135	♂	8	1	0.9433	0.8969	0.0464	4.9
136	♀	8	1	0.9271	0.8871	0.0400	4.3
137	♂	25	1¾	1.3590	1.2759	0.0831	6.1
138	♀	25	1½	1.2848	1.2031	0.0817	6.4
139	♂	90	2	1.7927	1.6598	0.1329	7.4
139			48	1.7927	1.4398	0.3529	19.6
140	♂	63	2	1.6050	1.4852	0.1198	7.4
140			48	1.6050	1.3611	0.2449	15.2

The specific gravity of B-15 is about that of the brain. Any loss of weight while the tissue is in the fluid is therefore due to loss of water. King ('10) found that the loss of weight during fixation in Ohlmacher's fluid varied from 8 per cent to 15 per cent, when left in the fixing fluid from 1 to 6 hours. The 8 per cent loss was on the brain left in 6 hours. She concludes that length of time in the Ohlmacher fixing fluid does not make much difference in the shrinkage. Her method of calculation, however, was to weigh the brain after fixation and compare this weight with the computed weight for a rat of this size and body weight.

The percentage losses of weight shown in table 3 run from 4.3 to 8.8 per cent when the tissue is left in the fixing fluid one to two hours, but jump to as high as 19.6 per cent when left in 48 hours. Age seems to make little difference, if any, when fixation time remains the same.

SHRINKAGE DUE TO ALCOHOL

It is well known that alcohol shrinks tissue. Table 4 shows that this is considerable if the tissue remains in the alcohol long, running as high as 17.6 per cent when left in 48 hours. These figures show the desirability of passing the tissue through alcohol as quickly as possible.

Table 4 shows also the total loss in weight due to fixing fluid and alcohol up to 60 per cent. These figures run on the whole a little less than those given by King ('10) for Ohlmacher fixation followed by 48 hours in 70 per cent alcohol, these running from

TABLE 4

Showing typical losses in weight of brains in alcohol after fixation as described in table 3, and the total loss of weight due to the effects of the fixing fluid and the alcohol, this loss increasing with the length of time the tissue is left in each fluid

NUMBER OF RAT	SEX	AGE IN DAYS AFTER BIRTH	NUMBER OF HOURS IN ALCOHOL	WEIGHT IN GRAMS WHEN THE ALCOHOL WAS STARTED DROPPING	WEIGHT AT END OF THE TIME INDICATED IN COLUMN 4	LOSS OF WEIGHT	PERCENTAGE LOSS OF WEIGHT	TOTAL LOSS OF WEIGHT IN FIXING FLUID AND ALCOHOL (60 PER CENT)	TOTAL PERCENTAGE LOSS OF WEIGHT
133	♂	2	2	0.2640	0.2360	0.0280	10.6	0.0500	17.4
133			48	0.2640	0.2294	0.0346	13.0	0.0666	23.6
135	♂	8	2	0.8969	0.8780	0.0189	2.1	0.0650	6.9
135			48	0.8969	0.7458	0.1511	16.8	0.0975	20.7
136	♀	8	2	0.8871	0.8664	0.0207	2.3	0.0607	6.5
136			48	0.8871	0.7309	0.1562	17.6	0.1962	21.1
137	♂	25	48	1.2759	1.1046	0.1713	13.6	0.2544	18.7
138	♀	25	48	1.2031	1.0145	0.1886	15.6	0.2703	21.1

16 per cent to 30 per cent, but most of them being in the neighborhood of 21 per cent. There is probably, then, very little difference, if any, between the shrinkage effects of these two fixatives and methods of preparation up to 70 per cent alcohol.

Some rough areal measurements of mesial views of brains indicate that the chief shrinkage is due to alcohol in the method described in this paper, taking the whole technique from fixation through paraffin to the slide. Some substance for replacing alcohol is therefore a decided need in technique.

CONCLUDING REMARKS

I have searched the literature for some mention of the use of chromic acid with a picro-formol-acetic combination, but find none. It would seem that my special contribution to microscopic technique as a result of these experiments is the fixative and the method of agitating fluids automatically as described in this paper.

I am greatly indebted to Professor McClung for constant criticism and advice; also for as constant encouragement when attainment of the desired result seemed hopeless. From him came the suggestion to add to the fixatives some urea, which had proved efficacious in some experiments carried on by Miss Eleanor Carothers at his direction. As an account of these experiments will be printed later by Professor McClung, no further reference need be made in this paper.

The Zoological Department of the University of Pennsylvania has provided me with laboratory facilities at Woods Hole and has placed its exceptional photographic facilities in the University at my disposal.

Professor Donaldson has shown a cordial interest in this work and his encouragement and advice have been very helpful. Wistar Institute has supplied the rats, the number of which passed the hundred mark, and has also provided equipment for carrying on these experiments.

GENERAL CONCLUSIONS ON TECHNIQUE

For cytological work, the slightest gain at any point in the technique is worth working for.

Very gradual change of fluids, agitation of the fluids during changing, and slow infiltration appear essential in order to get the best results from any fixative.

The addition of a low percentage of urea to fixing fluids results in sharpening the chromosomes and preserving the structure of the achromatic nuclear material. It may help penetration of the fluid.

Picro-formol-acetic mixtures are more effective when used at about 38°C. Cold is detrimental.

Flemming's fluid is more effective if used at 0°C. or a few degrees lower.

Flemming's fluid is of no value as a brain fixative at any temperature. It at times (if urea is added) isolates metaphase and anaphase chromosomes in spermatocytes somewhat better than any other fixative tried (except B-15), but is hard on the rest of the tissue and shrinks heavily.

Anilin oil is an excellent substitute for the higher alcohols.

Xylol shrinks tissue more than the vegetable oils.

Special conclusions applicable to the albino rat

B-15 is the only fixative tried which clearly differentiates brain chromosomes. It is superior to all others as a general fixative for developing and adult testis and central nerve tissue. It insures cytological detail in the testis better than any other.

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EXPLANATION OF PLATES

The figures are photographed from material fixed in five different ways. They show the essential differences between poor and good preparations.

Plate 1 illustrates the effects of fixatives upon the brain: Plate 2, upon the testis.

Modes of preparation. Figures 1, 3 to 7, 12, 14 and 17 were prepared by the method described in this paper. Figure 2 was fixed in Carnoy's fluid plus 1 per cent chromic acid and 2 per cent urea (table 2, no. 15), and carried through by the method described herewith. Figures 8 and 9 are from tissue fixed in Carnoy's fluid, dehydrated and double-imbedded as described by King ('10). Figures 10, 13 and 15 are from tissue fixed in Flemming's fluid and carried up by the method described in this paper. Figures 11 and 16 are from tissue fixed in Bouin's fluid and carried through by the method described in this paper. Sections were cut at 6 to 10 micra and stained with Iron Haem. and Or. G.

Photographing. Figures 1, 10, 11 and 12 were made with low-power projection lenses; figures 15, 16 and 17, with Zeiss 4 mm. apoc. obj. and Zeiss No. 4 projection ocular; figures 2 to 9, 13 and 14 were made with Zeiss 2 mm. oil immersion apoc. obj. and Zeiss No. 4 projection ocular. All were reduced for publication to the magnifications indicated below.

PLATE 1

EXPLANATION OF FIGURES

1 Sagittal section through cerebellum and medulla of 7-day rat. *Ext. gr. layer* is the region of actively dividing cells, magnified portions of which appear in figures 5 to 9. Only a small portion of the medulla is shown. × 15.

2, 3 and 4 show typical effects of different fixation upon cells in the medulla. Tissue is from 7-day rats. × 600.

2 Fixation: Carnoy plus 1 per cent chromic acid and 2 per cent urea.

3 Fixation: Picro-formol chrome-acetic in urea ('B-15').

4 Fixation: same as figure 3.

5 Dividing cell at inner edge of external granule layer of 7-day cerebellum shown in figure 1. Fixation by B-15. × 1200.

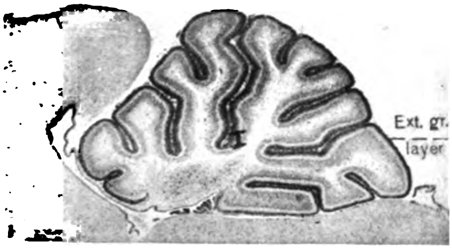
6 to 9 illustrate the external granule layer of the cerebellum of 7-day rat under different fixatives. The contrast is between the surface and the deep-lying portions. × 600.

6 Deep-lying tissue. Fixation: B-15.

7 Surface layer. Fixation: B-15. From same section as figure 6.

8 Deep-lying tissue. Fixation: Carnoy.

9 Surface layer. Fixation: Carnoy. From same section as figure 8.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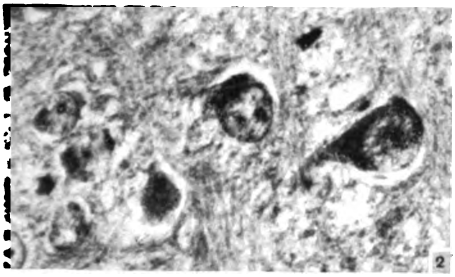
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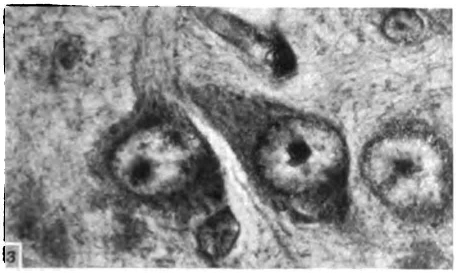
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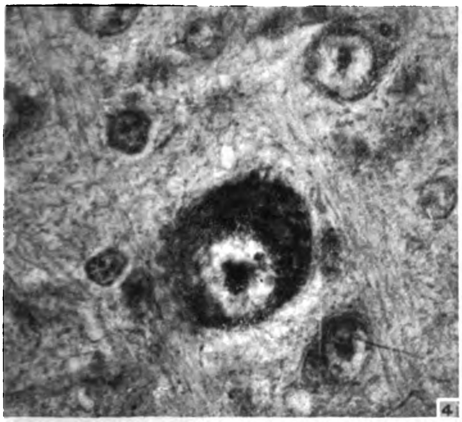
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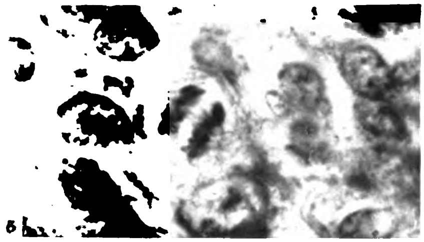
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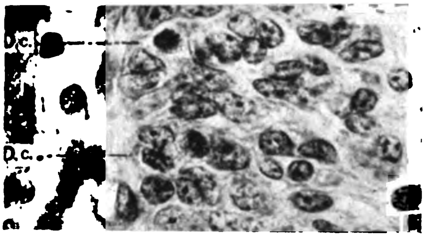
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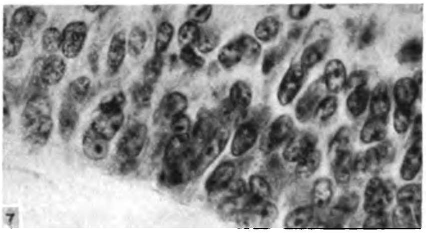
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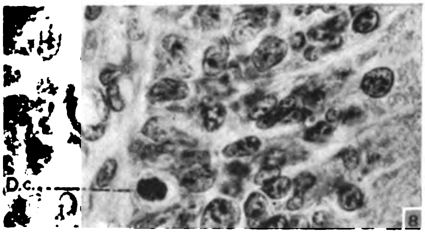
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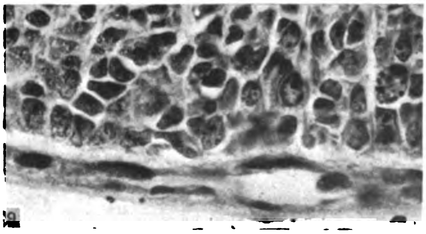
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PLATE 2

EXPLANATION OF FIGURES

10 Testis fixed in Flemming's fluid. × 24.

11 Testis fixed in Bouin's fluid. × 24.

12 Testis fixed in B-15. × 24. This tissue is from a younger animal than those shown in figures 10 and 11.

13 Same preparation as shown in figure 10. From adjoining slide. × 600.

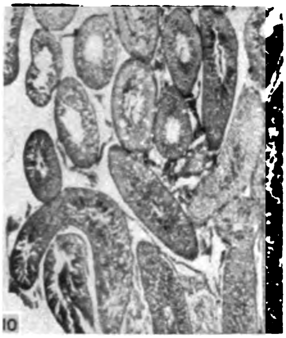
14 From same slide as shown in figure 12. × 1200.

14a This figure shows the cell directly above it in figure 14 at a different focus. × 1200.

15 From same slide as shown in figure 10. × 300.

16 From same slide as shown in figure 11. × 300.

17 From same slide as shown in figure 12. × 300. The chromosomes appear at some disadvantage in this figure compared with those in figure 15. The reasons for this are two: (1) they are lateral views in figure 17 as opposed to polar views in figure 15; (2) the cytoplasm is much better preserved in figure 17. A third reason may be suggested: they are less shrunken than in figure 15.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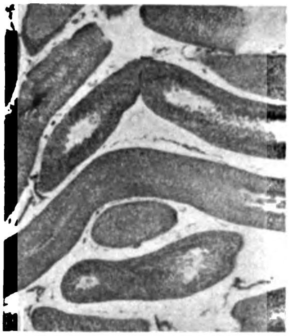

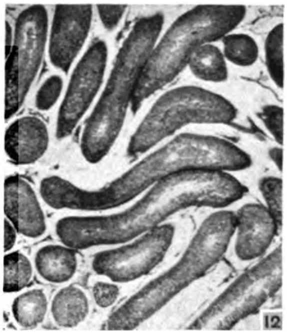
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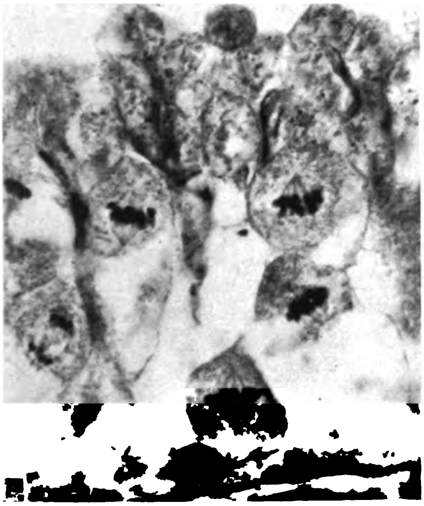

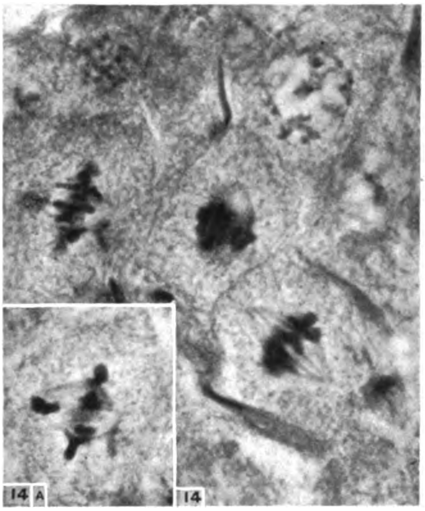
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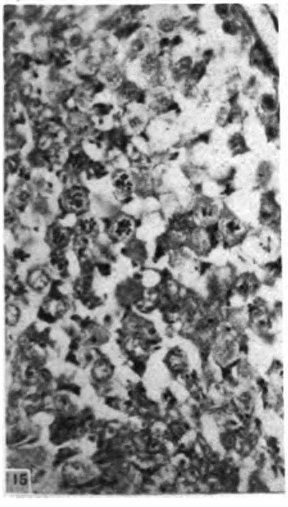
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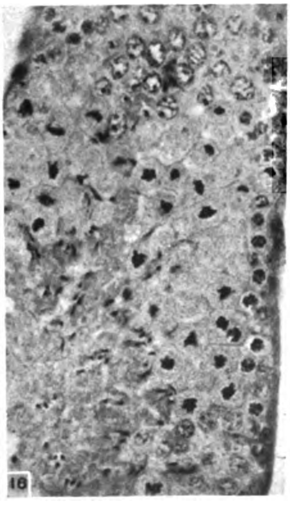
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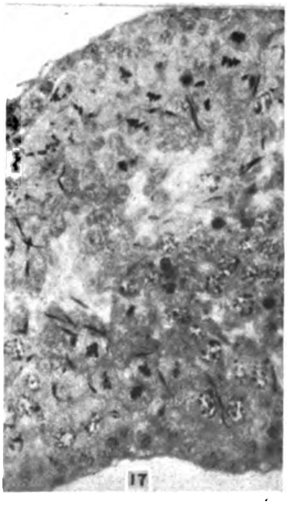
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Miscellaneous Notes on Odonata.

By Mary B. Lyon,

With Comments on the Dimorphism of the Females of Ischnura verticalis,

By Philip P. Calvert.

MISCELLANEOUS NOTES ON ODONATA BY MARY B. LYON.*

I. A note on the Emergence of Gomphines 57
II. The Nymph of *Enallagma ebrium* Hagen 57
III. The Supposed Dimorphic Female of *Ischnura verticalis* Say.. 59
Bibliography 62

In the course of an ecological study of the dragonfly nymphs of Cascadilla Creek, made at Cornell University in 1912-13, the results of which were published in the News for January, 1915, some observations on Odonata were made, which fell outside the scope of that work. These are here brought together and a description of the nymph of *Enallagma ebrium* Hagen is included.

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* Contribution from the Limnological Laboratory of the Department of Entomology, Cornell University.

I. A NOTE ON THE EMERGENCE OF GOMPHINES.

At the southern border of the Cornell University campus, a small stream known as Cascadilla Creek divides into two branches. After running through a grassy meadow these two branches again unite to form Dwyer's Pond. During the summer the upper third of one branch dries up, making a pond of the remainder of it. The other branch becomes a slow, sluggish stream. These quiet waters with their beds of soft mud and sand make an ideal home for the Gomphine nymphs which live there in great abundance. How great I did not realize until the morning of May 7, 1913, when the unusual number of birds along the banks attracted my attention as I came into the meadow. When I walked over to look I saw a great many Gomphine exuviae and closer examination showed many glistening wings among them. Along the banks of the stream and pond as far as six feet away from the water the ground was strewn with them. In a typical region I counted twenty-seven cast skins in a space only two feet square. Not a *Gomphus* was seen on the wing nor were any observed for several days afterward. The sandpipers, bronzed grackles, red-winged blackbirds, sparrows and probably other birds had enjoyed a sumptuous feast, as this was evidently the one morning of the season which hundreds of Gomphines had chosen for their emergence.

II. THE NYMPH OF ENALLAGMA EBRIUM HAGEN. NOTES AND DESCRIPTION.

In addition to the dense population of burrowing nymphs in this part of Cascadilla Creek there is another one composed chiefly of damselfly nymphs, which inhabits the waterweeds. One of the most numerous species of this group is *Enallagma ebrium* Hagen. Some of these nymphs taken into the laboratory and reared in a cylindrical wire gauze cage (Needham

'99) emerged June 20. Imagos appeared in the field a few days later and were common until July 30, although by July 4 the season of transformation was practically over. As is the custom of other Enallagmas, the male usually accompanied the female during oviposition. I saw only one female descend beneath the surface of the water. She was deserted by the male, who hovered over the water in an agitated manner for ten minutes. He flew to the shore, but returned again to remain twenty minutes longer above the spot where she had disappeared. On July 16 a pair was copulating on the Alumni Field about an eighth of a mile from Cascadilla Creek, the nearest body of water from which they could have emerged.

*Description of Nymph.** (Plate I, fig. 3, text figs. 1-4).—The nymph of ***Enallagma ebrium*** Hagen appears more active than that of other species of ***Enallagma.*** Even at transformation time the color is such a clear green that the nymph looks as though it had recently molted, but a very slight pigmentation sometimes darkens the shade.

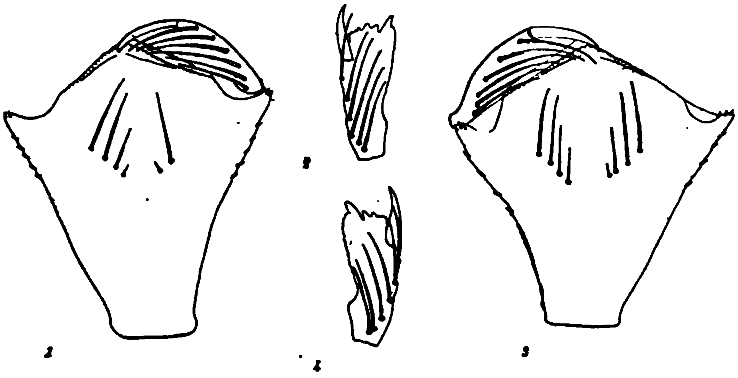

Fig. 1.—Labium showing the usual number of mental setae.
" 2.—Lateral lobe of labium shown in Fig. 1.
" 3 and 4.—Labium and lateral lobe showing the extra number of setae.

[*The nymph of *E. ebrium* has been described from a single specimen by Dr. E. M. Walker (Can. Ent. xlvi, p. 351, Oct., 1914) some months after the receipt of Miss Lyon's MS. by the News. Since Miss Lyon had a greater number of individuals at hand in drawing up her description and has accompanied it with figures, we publish her account as received. Plate I was published in the January number of the News.—Ed.]

Labium with hinge halfway between first and second pairs of legs. Mental setae variable in number, usually four on one side, two on the other. Inner seta of the four reduced to a mere spinule, as in the corresponding one of the two, while the one next to it may be nearly one-third shorter than the normal. Labium occasionally with four mentals, two of which are reduced in size, especially on one side. Lateral setae six. Lateral lobe with three distinct teeth above the end hook, preceded by three minute teeth between which project two spinules. Femora with dark subapical rings. Tibiae with dark basal ring. Gills lanceolate; widest just beyond the middle, with blunt tips; proximal half of their ventral margins straight and strongly spinulose; the dorsal margin slightly convex, somewhat spinulose; distal margin bearing a few long hairs. Gills hyaline with four or five wide, dark, lacey bands formed by pigment covering the tracheal tubes.

Dimensions.——Body 12-13 mm.; gills 5.5 mm. additional.

III. THE SUPPOSED DIMORPHIC FEMALE OF ISCHNURA VERTICALIS SAY.

The dimorphism of the female of various species of *Ischnura* has been generally accepted, two forms having been described as the orange and black females of *Ischnura verticalis* Say in the keys of Calvert ('93), Williamson ('00), and others. According to Williamson, both become pruinose, the black female less so than the orange. He also notes that the pruinose orange females are more numerous in the spring than the bright orange or black ones, while the black females are most numerous in the autumn. Since it seemed probable that these three forms might be different stages in coloration due to age, some experiments were undertaken to determine their relationship if possible. The results have proved that there is but one female for *Ischnura verticalis* Say.

Apparatus.—The banks of Cascadilla Pond are straight and grass-grown. Into one of these banks a cut was made so that about ten inches of water might run into the bay thus formed. A simple cage was built to cover this bay together with a bit of the bank. The framework was made by crossing two pieces of heavy wire to form the diagonals of this rectangular space. The wires were bent at the corners so that when placed firmly in the ground they made a framework ten inches high. Over this support a covering of white mosquito netting was stretched, and above that a layer of cheese-cloth. The edges of both were fastened by stones firmly to the ground on three sides, that of the fourth being held down by the weight of the water into which it extended. A natural habitat was thus secured with grass, water, plenty of room for flight, and sunlight a part of the day.

Experiment.

4 p. m., July 11. Eight orange females and four males of *Ischnura verticalis* Say which had emerged in the laboratory July 9 and 10 were placed in the cage. The length of the abdomen of the females was noticeably different and when measured was found to vary from 20.5 mm. to 23 mm., the hind wing varying in length correspondingly. Each day the grass was swept with an insect net, and when the damselflies were removed the catch was put into the cage to supply food. The females were examined daily and the colors noted. Two orange females died and one was drowned within the first four days.

4 p. m., July 17. One female showed the first indication of change, the bronze black of its abdomen beginning to look dull and velvety.

9 a. m., July 18. The female noted on July 17 was found dead in the water. Three orange females now remained. On one the black was dull and velvety and the under parts of the head and thorax were greenish.

3 p. m., July 19. Two females were entirely pruinose and agreed so closely with Williamson's description of the living black female that I can do no better than to quote it. "Bluish or grayish, pruinose; mid-dorsal thoracic and humeral stripes black; abdominal segments 1 to 7 with their apices darker, usually black, and 8 to 10 black." The marking on segment 8 was a black triangle covering the posterior half of the segment, with the base of the triangle at the apex of the segment. The face, sides of the thorax and under parts were greenish. The pterostigmata of all four wings were brown. When placed in alcohol this specimen agreed entirely with Williamson's description.

At this time, the remaining bright orange female was found mating.

9 a. m., July 20. The orange of the female which was seen mating on the previous afternoon had changed to dark brown on the thorax and head, but a little orange was still visible on the sides of segments 1 and 2. The remainder of the dorsum was dull grayish blue and there was a band on the apex of segments 1 to 7 and the posterior half of 8, while all of segments 9 and 10 were blackish. The face, sides of the thorax and the underparts were greenish yellow.

The two pruinose females were slightly darker in color, the apex of the blackish triangle on 8 having almost reached the basal border of the segment. These three individuals were placed in a smaller cage of wire gauze.

Six orange females which had emerged in the laboratory on July 19 and 20 were now placed in the large cage and no males included.

9 a. m., July 24. No change occurred until this date, when one orange female became slightly pruinose with a tinge of brown on the abdomen.

July 25. Two other females had become pruinose, and in time these and the one observed July 24 assumed a coloration like the one first described. No further change was noted in those which had been placed in the small cage except that they became more pruinose.

From these data we may conclude that the female of *Ischnura verticalis Say* varies somewhat in size and at the time of emergence is marked with bright orange on the postocular spots, the thorax and the first three segments of the abdomen. Four days or more after emergence, this orange turns to brown and then to a gray blue. At the same time the bronze black becomes bluish or blackish. The face, sides of the thorax and the underparts become yellowish green. This entire color change may take place in less than eighteen hours, and may occur either before or after mating. The rapidity with which the color changes occurred after mating in the instance observed may account for the fact that no orange females have been seen ovipositing in the field. The fact that the pruinose orange female assumes with age the deeper coloration of the so-called black one, would naturally make this latter form far more numerous in the fall.

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THE DIMORPHISM OR DICHROMATISM OF THE FEMALES OF ISCHNURA VERTICALIS.

By PHILIP P. CALVERT, University of Pennsylvania, Philadelphia, Pa.

Of the authors who have written on the females of *Ischnura verticalis,* I believe that only Calvert (1893) and Williamson (1900), both in part only, Ris (1903) and Walker (1908) have correctly recognized the true dimorphism which exists in this sex ***in individuals of the same age.*** The two forms recognized by others are ***not forms of equal age, but color differences due to age.*** Miss Lyon, in her preceding paper, has shown that the orange female becomes pruinose and black, but I think that she has experimented with but one form of female to be found in this and other species of *Ischnura,* that which in treating of the genus, in the *Biologia Centrali-Americana,* I called the ***heterochromatic*** female, as being colored unlike the male.

The two true dimorphic or dichromatic forms are only to be recognized with certainty when the student has teneral, or relatively recently emerged, females before him. Their differences may be most concisely shown in a table:

DIFFERENTIAL CHARACTERS	HOMOEOCHROMATIC, "BLUE" OR "GREEN," ♀	HETEROCHROMATIC OR "ORANGE," ♀
Pale postocular spots.	Not confluent with the pale color of the rear of the head.	More or less confluent with the pale color of the rear of the head.
Predominant color on thorax.	Pale bluish.	Yellow or pale orange.
Blackish humeral stripe.	Wider than the pale antehumeral stripe.	Narrower than the pale antehumeral stripe.
Abdominal segment 1: Dorsum.	Dark metallic blue or green, only the articular membrane between 1 and 2 pale.	Yellow or orange with small black dots (four, two or one).
Sides	Pale bluish.	Yellow or orange.
Abdominal segment 2: Dorsum	Dark metallic blue or green for its entire length, narrowed at its hind end.	Yellow or orange with black markings varying from a narrow crescent at two-thirds of the segment's length to a spot on the first- and one on the third-third, connected by a fine mid-dorsal line.
Sides	Pale bluish.	Yellow or orange.
Abdominal segment 3: Dorsum	Dark metallic blue or green contracted at anterior end to a mid-dorsal line (thus forming what is usually described as a basal pale ring interrupted mid-dorsally) and at the hind end to a smaller degree.	Yellow or orange, the hindmost fourth dark metallic green prolonged forward as a tapering black line almost to, or to, the fore end of the segment.
Sides	Pale bluish.	Yellow or orange.

Both of these forms have, in their earliest imaginal state, *the eighth and ninth abdominal segments pale blue,* each segment *with a lateral black stripe* each side, extending from the fore end to two-thirds of the segment's length on 8, and to half-length of 9. The right and left lateral black stripes are connected with each other on each segment by a transverse black stripe at the fore end, which cross-stripe is distinctly wider (equal in some to even one-fourth of the segment's length) on 8 than on 9 (on the latter a little more than a line).

There is no constant difference in the size of these two females.

The homoeochromatic female from which I have chiefly drawn up this description appears, from its chitinization, to be more immature than the heterochromatic female which I employed. There is, therefore, no ground for supposing that the former is an older and in consequence a more blackened form of the latter. Judging from the material before me, both the homoeochromatic and the heterochromatic females above described, become darkened and eventually pruinose. One can, in some cases, determine whether a partly blackened female belonged originally to one or the other form by examining carefully those parts compared in the table on page 63 and recognizing their shape, size or tint under the partial concealment of the changing colors. Probably the pale blue on abdominal segments 8 and 9 disappears earlier in the heterochromatic females than in others.

A hint that occasionally an intermediate or a composite of the two forms exists is afforded by a well-chitinized female taken by myself at Delair, New Jersey, August 13, 1903. In most respects it agrees with our table for the homoeochromatic form, but differs therefrom in having the pale post-ocular spots confluent for about one-third of the width of each with the pale color of the rear of the head, the sides of abdominal segment 3 with an orange tinge which invades the dorsum in the anterior two-fifths of the segment, leaving only a narrow mid-dorsal black line on this part of the segment and two or three small isolated black spots on the sides, the posterior three-fifths of the dorsum being solidly metallic greenish-black.

Material examined in drawing up the preceding account:

Homoeochromatic Females.—Tinicum Is., Pennsylvania, June 22, 1889, 1 teneral; west side Schuylkill River below Gray's Ferry, Philadelphia, May 4, 1889, 1 teneral. Philadelphia, June 5, 1900, one specimen a little older. Philadelphia, May 22, 1904, 1 teneral. These four have abdominal segments 8 and 9 blue with lateral black stripes. Tinicum Is., May 21, 1904, one specimen with abdomen pruinose.

Heterochromatic Females.—Tinicum Is., Pa., June 22, 1892, 1 teneral; May 21, 1904, two specimens. Christiana Creek, Elkhart, Indiana, May 21, 1897, 1 teneral by R. J. Weith. Vermont, by Mrs. A. T. Slosson, 1 specimen. Only the Tinicum Is. specimens have 8 and 9 blue with lateral black stripes, but all five have yellow or orange distinct on head, thorax and first three abdominal segments.

Composite (?) *Female.*—The Delair female noted above.

The eleven specimens cited are in the collection at the Academy of Natural Sciences of Philadelphia. At different times I have given other specimens to Mr. E. B. Williamson and Prof. J. G. Needham.

Among my unpublished notes I find the following which I leave in their original form:

Teneral female *Ischnura verticalis* taken at pond, Botanical Garden, University of Pennsylvania, 8.55 A. M., May 13, 1904; able to fly, wings and body still quite flabby, colors pale but sufficiently well-marked to see that the 8th and 9th abdominal segments were pale with a black stripe on each side of each as in the male of this species. Placed in a "Lightning" pint jar whose top was covered with a piece of netting; contained a bit of twig for fly to crawl on.

Examined 3.30 P. M. same day; dark colors much more marked, pattern of 8 and 9 as before.

May 14, 9.45 A. M. Pattern as before, dark colors on body generally well-marked, black stripes on sides of 8 and 9 not reaching to apex of respective segments.

May 15, 12.30 P. M. Markings as on preceding record.

May 16, 12.30 P. M. No apparent change.

May 17, 9.15 A. M., do. May 18, 6 P. M., do. May 19, 6 P. M., do. May 20, 1.45 P. M., do. May 21, 3.45 P. M., do. May 22, A. M., dead, no change in the colors but still evidently teneral, no pruinosity.

Two teneral females of *Ischnura posita* collected same time [*i. e.*, May 13, 1904, etc.] and place. If anything, more teneral than *I. verticalis* ♀; did not show any trace of pale markings on dorsum of 8 and 9, *i. e.*, had practically pattern of adult. Three males, one female, teneral, *posita*, taken 10 A. M., May 14, 1904, likewise did not show any traces on 8-10 of a pattern different from that of the mature individuals.

It is probable that the teneral female of *I. verticalis* of May 13-22, 1904, is the homoeochromatic female cited above as of May 22, 1904, although it is merely labeled "Phila., May 22, 1904." Although it was kept alive in captivity for at least eight days, there is nothing in the notes to indicate that it was given any food and in this respect, and in its more limited range of movement, the experiment differed from those by Miss Lyon. These differences may account for the absence of color changes.

The authors who have described the females of *Ischnura verticalis* are the following:

Say (1839) described a "slightly pruinose" female, but it is not possible to determine whether it was of the homoeochromatic or heterochromatic form. In the original description, the signs ♂ and ♀ have evidently been transposed, as the content and the first line of the account of the true male indicate. LeConte (1859) in his edition has retained these signs and has made the error worse by altering the word "female" to "male" in the first line of the description of the true male.

Hagen (1861, page 76, under *A. ramburii*) very briefly described the heterochromatic female of such an age that "the whole of the abdominal dorsum" had become "brassy-fuscous" and a still more aged "pruinose" female which may have been originally homoeo- or heterochromatic.

Selys (1876) described an "adulte" and a "jeune" female, but there is nothing in the descriptions to indicate to which of the forms we have distinguished above they belonged.

Provancher (1876) translates Hagen's description of 1861 under the same name, *Agrion ramburii.*

Calvert (1893) distinguished a "black" and an "orange" female. Under the former heading he mentioned one as being "colored like ♂ (teneral)"; this is the homoeochromatic female of the present paper and some of the specimens on which this statement of 1893 was based are still before me. The other females referred to as "black" were said to have the "greater part of body *pruinose,*" etc.; this would apply equally well to aged homoeochromatic, or to aged heterochromatic, females. The "orange" females of 1893 were evidently heterochromatic forms in which the blue of abdominal segments 8 and 9 had already been replaced by black.

Needham (1898) figured a heterochromatic female of the same degree of coloration.

The two females described by Kellicott (1899) are, following his order, "(b) orange and bronze black" = a heterochromatic female as described by Calvert (1893), and "(a) black and green (pruinose)" = an aged homoeo- or heterochromatic female.

Williamson (1900) recognized a "black" and an "orange" female. As the black females are said to have "postocular spots connected or not," it seems likely that they included advanced individuals of both the homoeochromatic (*i. e.*, postocular spots not connected) and the heterochromatic (*i. e.*, spots connected) forms. His orange females are evidently heterochromatic forms like those of Calvert (1893) and of Kellicott (1899), but he perceived and recorded that "this form becomes entirely pruinose."

Ris (1903) regarded the females as showing "a curious polymorphism." His expression "In some teneral females of the green form the upper side of segments 8 and 9 is largely blue" refers to homoeochromatic females of *verticalis* or of *kellicotti*, more probably the former. While he does not mention the earliest stage of heterochromatic females (*i. e.*, those with blue on abdominal segments 8 and 9), he recognizes the dimorphism (or dichromatism) of green and orange females, although his "fully adult" individuals of the latter are not the most fully altered in their colors.

Needham (1903, pl. 17, fig. 5) has reprinted his figure of 1898.

Two figures by Howard (1903, pl. xlvii, figs. 2, 4) seem to be referable to pruinose females of indeterminable form.

Walker (1908) briefly summarizes "two color varieties" of females, "the 'black female,' colored like the male when young," which implies our homoeochromatic form, and the "orange female," a heterochromatic form of unspecified age. He adds, "Both forms become pruinose when old, appearing then as though covered with a dull bluish dust . . . The latter [black female] seems to become pruinose very soon after maturity." The last-quoted sentence probably explains why so relatively few homoeochromatic females with blue on segments 8 and 9 have been recorded in the literature, and why the true characters and relations of this form have not been more largely recognized.

Muttkowski's descriptions of 1908 apply to the same conditions noted by Kellicott (1899).

If my interpretation of specimens and of descriptions be correct, I must assume one of three possibilities from Miss Lyon's work. 1. That she overlooked the presence of blue with the lateral black stripes on abdominal segments 8 and 9 of her youngest orange females, or 2, that the blue on these segments disappeared within 48 hours, or 3, that some orange females never, at any time, possess this coloration on the two segments in question. The determination of the correctness of these assumptions affords a field for further observation and experiment. Those who undertake this should carefully note the colors of the living insects at frequent intervals with reference to some work like Ridgway's *Color Standards and Color Nomenclature.*

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Studies on Costa Rican Odonata.

VI. The Waterfall-Dwellers: The Transformation, External Features and Attached Diatoms of Thaumatoneura Larva.

By PHILIP P. CALVERT, PH.D., University of Pennsylvania, Philadelphia, Pa.

(Plate XI)

In number V* of these Studies we have described the adults of *Thaumatoneura* and some of their habits. While we observed motions on the part of females which, by analogy, we interpreted as those of oviposition, we were never able to detect the eggs or to obtain the young larvae from pieces of roots and of moss in which we supposed eggs to have been placed. We were more successful with older larvae. On June 26, 1909, a few were found in among the roots that hung in front of the rock of the farther, or lower, waterfall at Juan Viñas, where they were constantly bathed in the water. On June 27, 1909, while hunting over the rocks at the foot of the higher, or nearer, waterfall, we found a large larva of the same kind (No. 4 of the list on page 300) "crawling up out of the water on to a big rock. This being a hopeful indication of near transformation we secured him and carried him back to the cabin where we rigged up an ingenious and home-like stream for him in the fixing dish aided by a big stone and a bottle." All these went to our headquarters at Cartago but all died before they yielded the wished-for imago. We write of them now as *Thaumatoneura* larvae by reason of proof acquired ten months after their discovery. *Then* we had only the presumption based on their occurrence at the same localities with the winged individuals. On August 2, at the nearer waterfall, we found an exuvia which we took to be that of *Thaumatoneura*. Search for larvae at the farther waterfall on December 2 was unsuccessful. On March 23 and 26, 1910, larvae were not rare on the rock face of the nearer waterfall where it was kept wet by the spray and, on the 26th at least, where there was a thin layer of organic mud on the rock surface. A visit to the same fall on April 24 yielded twenty-two exuviae, but none of them had an imago with it. They were chiefly on the rock face of the waterfall but in spots more or less protected from the falling water. Smaller larvae than those indicated by the exuviae were crawling over the rocks.

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* Ent. News, xxv, pp. 337-348. Oct., 1914.

This waterfall was nearly vertical in its descent from the top of the canyon of the Rio Reventazon but, at about one hundred feet above the railroad track (which occupied a narrow ledge cut out of the cliff face), the water usually disappeared beneath a mass of boulders and flowed underground through loose soil to reappear on the slope far below the track. Up over these boulders we climbed to the foot of the actual fall with its vertical rock face. The amount of water over and among the boulders varied with the rains. On April 26 we ascended with more difficulty than usual, owing to the greatly increased flow of water which covered many of the usual footholds. Mrs. Calvert, who was in advance, found a large larva on the wet slippery face of the fall itself in such a position that a small projection above it gave it shelter from the direct spray. This larva we saw transform. She noted at the time: "Unlike other dragonflies we have watched transform, the wings while they hang limp and wet, in fact until they are full size and dry, are green. The body of the dragonfly is brown and green and as the creature hangs on the rocks, the body and wings giving the little trembling jerks that usually accompany the growing or 'filling out' of a transforming dragonfly, the likeness to a green leaf on a stem trembling in the breeze is very marked. There is a great deal of a small-leaved *Commelina* on the face of the fall as well as some delicate grasses, both of a bright pea-green, and we ourselves frequently confused the dragonfly we wanted to watch with one or other of these if we happened to move our eyes from it. It certainly seems to be a good case of protective coloring." Her record of the progress of transformation follows:

10.30 A. M. Head and thorax free dorsally.

10.35. Antennae and head entirely free, wings beginning to be withdrawn.

10.37. Front wings free.

10.37.40. Hind wings and first legs free.

10.38.20. Second legs and abdominal segments 1-3 out.

10.39.30. Third legs out, wings bright yellow, front edges and principal veins black, eyes almost black, body pale green chiefly. Resting period until

10.49, when imaginal legs grasped larval skin; very slender tracheal linings still connect exuvia and thoracic spiracles; width of head of adult much wider than head of exuvia.

10.59. Abdominal segments 4 and 5 free.

11.00. Sixth abd. seg. free.

11.00.10. Seventh; 11.00.15. Eighth; 11.00.40. All abdominal segments free. Wings still of exuvial length. Abdomen pale green, four longitudinal blue-brown lines on dorsum and spot on middle of lateral carina of each abdominal segment, appendages pale yellowish green. Following bluish-brown, labrum, clypeus, vertex, marks on middle prothoracic lobe, paramedian line and broad antehumeral stripe on mesepisternum, posthumeral stripe, double line above metastigma.

11.03. Wings lengthening, hind to end of abdominal segment 5.

11.11. Left hind wing as far as hind end of abdominal segment 8, the other three wings to hind end of abd. seg. 7.

11.13. Left hind wing to hind end of abd. seg. 10, the other three wings to segment 8. All wings pale pea green, veins blackish.

11.16. All wings longer than body plus appendages and subequal.

By 1 P. M. the green color had left the wings—how long before we do not know.

This (male) eventually colored as *Thaumatoneura inopinata* and, with other specimens, was left to dry in a paper bag, from which it was set free by some meddler on April 28 and so lost. Its exuvia, however, was carefully preserved and labeled at once. On the same morning and at the same place another larva was found transforming and yielded a male of *Th. pellucida** so that our previous surmises as to the identity of these larvae were established as definite facts.

On April 30 we went again toward the higher waterfall but, when we reached the mass of boulders below it, found that a landslide had occurred changing their positions very greatly. We ascended a short distance to reconnoitre and saw that some small trees had fallen, blocking our usual path, while rocks larger than one's body had been rolled down, many of them being quite unstable. In fact the change since the 26th, when we were here, was far greater than at any time during the preceding ten months in which we had known this fall. We concluded not to ascend on account of the risk involved and went on to the farther waterfall where two good-sized *Thaumatoneura* larvae were obtained, one of which transformed as a female before we left the fall. We subsequently learned that on April 28, when we were in a different locality, two distinct falls of rock, with the sound of crashing of tree branches, were heard in the direction of the higher waterfall, accompanied by the odor of newly-disturbed forest earth. It was our good fortune that this landslide did not occur until after our ten months' search had been crowned with success and we could smile at the efforts of the Genii of the Fall to veil the secret of *Thaumatoneura* from us.

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* In discussing the possible male dimorphism in *Thaumatoneura*, reference was made (Ent. News, xxv. p. 345) to similar phenomena in other animal groups. Since that article was published, Dr. T. S. Painter, of Yale University, has called my attention to the dimorphism of the males of *Maevia vittata*, a jumping spider. See his paper in Zool. Jahrb. Abth. Syst. Geogr. Biol. xxxv, pp. 625-636, 1913.

To make our larval record complete—one was secured on May 1 at a third waterfall, near the iron bridge over the Rio Reventazon, 800 feet below the railroad.

The habit of dwelling in and around waterfalls and cascades which we have described for *Thaumatoneura,* larva and adult, and which is also the habit of *Argia talamanca* associated with it, does not appear to have been mentioned for any other Odonata. Yet it is likely that such a habit will be found to exist in some other members of this group of insects, especially in the tropics of the Old World, where topographic and climatic conditions similar to those of Costa Rica prevail. In countries where frost occurs the low temperatures incident to this phenomenon may prevent the survival of Odonate larvae in waterfalls of small volume, where the water is spread out in a sheet of but a few centimeters in depth, as is the case in those falls which *Thaumatoneura* inhabits. On the other hand, the force exerted by falling water in *large* volume may be too great to permit Odonate larvae dwelling therein.

DIATOMS ATTACHED TO THE LARVAE.

While examining the larvae and exuviae of *Thaumatoneura* the great number of diatom shells attached to them was very apparent. A single leg, which had been detached from an exuvia, was sent to Prof. Albert Mann, Custodian in charge of the Diatom Collection of the U. S. National Museum at Washington, who has sent the following remarks on the diatoms adhering to this member:

> The species discovered on this very minute quantity of material are as follows:
>
> *Achnanthes ventricosa* Ehr.
> *Cocconeis placentula* Ehr.
> and variety.
> *Cymbella ventricosa* Ag.
> *Epithemia gibba* (Ehr.) Kg.
> *E. gibberula* (Ehr.) Kg.
> *E. zebra* (Ehr.) Kg.
> and variety.
> *Eunotia monodon* Ehr.
> *Gomphonema parvulum* Kg.
> *Navicula viridis* Kg.
> *N. oblonga* Kg., var.
> *N.* n. sp.
> *Nitzschia* sp.
> *Pleurosigma* sp.
> *Synedra ulna* (Nitz.) Ehr.
>
> Of the foregoing, *Epithemia zebra* is by far the most frequent, *Gomphonema parvulum* next, *Synedra ulna* next, *Achnanthes ventricosa* next, and the others rather infrequent. The unnamed *Nitzschia* and *Pleurosigma* afforded only one specimen each and their position was such that I was unable to correctly identify them, but I am quite confident that they are not new. The new *Navicula* is extremely small and very delicately marked, and I fortunately have two specimens.
>
> I should say that there were at least 500 diatoms on the small leg of this larva. The most of these are forms that grow attached by a transparent jelly-like stipe; but several of them are free and free-moving—for example, the different species of *Navicula*.
>
> The finding of these forms on this aquatic larva is of importance because it helps to throw light upon a difficult problem connected with the distribution of diatoms. It has always been more or less of a mystery how isolated pools and bodies of water of recent origin become stocked with a rather extensive diatom flora and frequently within a very short period of time. Little is known regarding the spore formation of diatoms, investigations in this respect having been confined almost exclusively to pelagic marine forms, such as *Rhizosolenia*. In most of the genera no spore formation has ever been discovered. Some light has been thrown upon this distribution by supposing these organisms are carried on the feet of wading birds, but this method of transportation is plainly very inadequate. If, however, we add to this transportation by aquatic insects, many of which have the power of flying, we have

a means of transportation that goes a long way to explaining the sudden appearance of large numbers of diatoms in new localities.

I therefore look upon this rather abundant flora on the minute leg of this aquatic insect as of some scientific importance. Of course, in this case the power to fly does not exist until a later period of development, but many of the water beetles and other insects are doubtless coated with living diatoms in the same way.

A reference to Van Heurck's *Treatise on the Diatomaceae* (English translation by Baxter, London, 1896) shows that most of the diatoms listed above, and especially those of most frequent occurrence, are "common everywhere," at least throughout Europe and, as they occur in Costa Rica also, probably throughout the world. It is of interest, moreover, to compare the data on the diatoms found attached to *Thaumatoneura* larvae with those relating to the diatoms enumerated, as occurring on the nymphs of various Odonata of the United States, by Miss Lyon in the NEWS for January, 1915, pp. 4-6.

DESCRIPTION OF THE LARVAE.

Material Studied. Larvae in alcohol:

(*a*) From hanging roots in farther waterfall, Juan Viñas, 3300 feet (1000 metres), June 26 and 27, 1909. Nos. 1, 2, 4 and 5, males, No. 3, female. (For No. 4, see Pl. xi., figs. 2, 3.)

(*b*) From nearer waterfall, Juan Viñas, 3400 feet (1036 metres), March 23 and 26, 1910. Nos. 6 to 8, females. (For No. 8, see Pl. xi, fig. 1.)

(c) From the same waterfall, April 24, 1910. No. 9, ♂.

[All of these died in attempted rearing. The following *Thaumatoneura* larvae are recorded in our rearing list and, as there is no entry of their having died, were presumably lost in the earthquake at Cartago, May 4, 1910:* No. 76 (one), No. 77 (one), No. 78 (two) all from the nearer waterfall, Juan Viñas, March 26, 1910.]

Exuviae:

♀ Nearer waterfall, as above, Aug. 1, 1909.

♂ *inopinata* same locality. April 26, 1910. (Watched in transformation.

♂ *pellucida* same locality, April 26, 1910. (Watched in transformation.)

17 ♂ ♂, 9 ♀ ♀ same locality, April 24 and 26, 1910, in various degrees of preservation.

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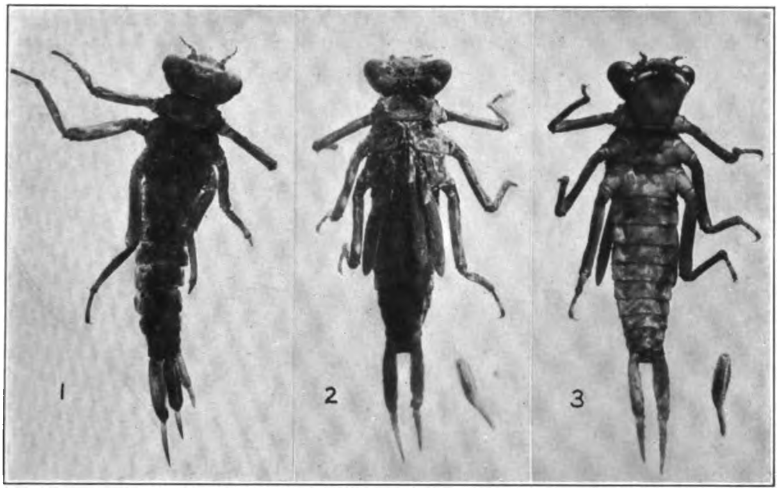
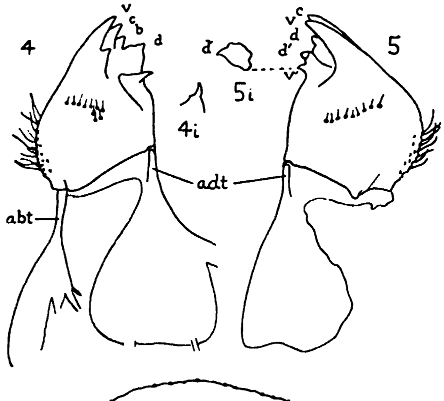
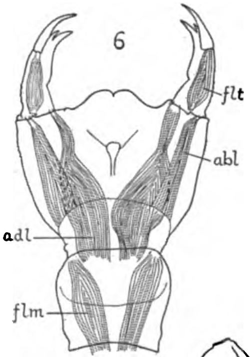
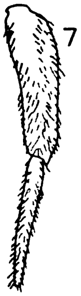
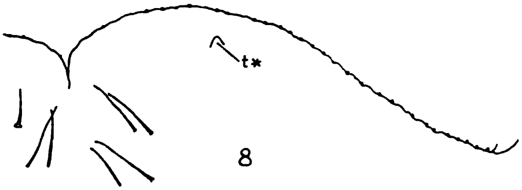
* See Ent. News, xxi, p. 336. July, 1910.

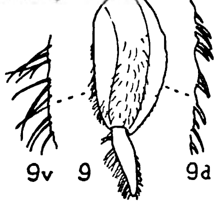












THAUMATONEURA LARVA—CALVERT.

The exuvia of April 30 was probably lost in the earthquake.

The following description is based on all the larvae studied and on the two exuviae of *inopinata* ♂ and *pellucida* ♂. The differences shown by the larvae of various sizes are given in the table on page 304. As has already been stated,* I have not been able to find any differences between these two exuviae other than those due to injuries sustained by the larvae previous to transformation.

Head shallowly concave posteriorly. Compound eyes and their ommatidial lenses very distinct; in most of the alcoholic larvae the dorsal surface of the eye below the chitin showing dull greenish-blue with 9 to 11 diverging darker streaks which radiate from a point caudad to the inner posterior dorsal angle of the eye. Ocelli indicated by three smoother patches amid the otherwise finely wrinkled and finely spinulose dorsal surface of the head. Nasus of the larvae with four pale streaks extending from its superior to its inferior margin, two of them submedian, the other two lateral, right and left. Labrum hardly notched on its free margin, which is obscured by matted hairs in the exuviae, especially at each side where, viewed from above, they form a small tuft. Edges of the genae which bound the eyes posteriorly and inferiorly with a row of closely-set spinules.

Antennae 7-jointed, decreasing in diameter from 1 to 7, lengths of the joints in larva No. 4: (1) .64, (2) .48, (3) .4, (4) .224, (5) .224, (6) .16, (7) .064, total 2.192 mm.

Mandibles stout. Right mandible (Pl. XI, figs. 4, 4*i*) at its apex with four teeth, of which the most dorsal or foremost is usually the widest, the second the smallest, the third and fourth longer than the other two, the fourth longest and having on its inner ventral edge a small basal denticle: on the inner surface, proximal to and distinctly separated from these apical teeth is a shorter, tapering and curved process terminating in an acute apex and bearing a minute anteapical posterior denticle. Left mandible (Pl. XI, figs. 5, 5*i*) with apical teeth similar to those of the right, but the foremost (most dorsal) tooth has a small dorsal denticle (except in larva No. 1) and the fourth (most ventral) tooth has no basal denticle and in some is no longer than, or even not quite as long as, the third tooth; the process on the inner surface is much larger antero-posteriorly than that of the right side, forming when viewed from below (the mandibles being open) a crescent-shaped ridge whose edge is crenulate or denticulate and terminates at both anterior and posterior ends in a slender acute spine; the concavity of the crescent is proximal. These processes of the inner surface of each mandible appear to correspond with the inner branch of the mandible of the larvae of *Cora*.† The lateral surface of the base of each mandible is armed with small spinules. Near the middle of the ventral face is a row or group of about ten setae.

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* Ent. News, xxv, p. 345. Oct., 1914.

† *Cf.* Ent. News, xxii, p. 52, pl. II, fig. 16.

Maxillae similar to those of *Cora*,† the attenuate tip of the inner lobe with one immovable tooth and two more proximal movable spines. There is a membranous supralingua between each maxilla and the hypopharynx proper, not visible in the exuviae.

Labium (Pl. XI, figs. 6, 8) when at rest reaching caudad to the level of the bases of the prothoracic legs but not as far as that of the hind ventral prothoracic margin. Median (mental) lobe with the distal margin 1.2 times as wide as its mid-longitudinal length, produced distinctly distad in the middle and there bilobulate, but the emargination which separates the lobules not reaching proximad as far as the level of attachment of the lateral labial lobes; a minute tooth on the inner dorsal surface of each lobule a short distance within (proximal to) the distal margin and a greater distance from the median line; this is t^* of the larva of *Cora* as shown in fig. 28, pl. III, *l. c.*, and the "pronounced tooth . . . on the sutural line" of the labium of larval *Lestes* (BUTLER)‡; distal margin minutely crenulate for its entire length, a very short seta between each crenulation and the next, lateral margins spinulose near the articulations of the lateral labial lobes; mental setae usually absent, but two pairs of short setae have been observed on each side of and near the emargination separating the two lobules of the distal margin. Lateral labial lobes spinulose laterad near base, without setae, each with a long curved tapering terminal spine ("movable hook") and two stout distal teeth, both shorter than the terminal spine, the inner (mesal) of the two longer than the other; three distal teeth exist in the larva of *Cora*, the most internal of which appears to be absent in *Thaumatoneura*.

Dorsal surface of *prothorax* sharply defined on all sides by a carina having a hexagonal outline (counting the curved anterior and posterior edges as but one each). Propleuron bounded inferiorly by a carina ending anteriorly in a spinulose tubercle. Mesostigmata very distinct, on transverse tubercles, open in larva No. 4 and probably in the exuviae, not open in the other larvae. Mesopleuron with a marginal carina. Metastigmata indicated, small, apparently not open.

Legs moderately long, moderately slender, each coxa with a superior spinulose tubercle, three tarsal joints, two curved simple tarsal claws, empodium present.

Abdomen triangular in cross-section, but the mid-dorsal angle rounded, each segment wider than high; of 10 complete segments which decrease in width very gradually and slightly from 1 to 5, more markedly from 6 to 9. Segment 1 with a short transverse row of setae

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† *L. c.* p. 53, pl. III, figs. 29, 31.

‡ Trans. Amer. Ent. Soc., xxx, p. 114, 1904.

on the mid-dorsum at about three-fifths of the segment's length; on 2 to 5 this row is represented by two separated patches of setae, one on each side of the mid-dorsal line and at the same part of each segment's length; on the dorsum of 6 to 10 are also similar setae but apparently, in most specimens, not as regularly arranged as on 1 to 5; in addition to the setae 1 to 10 bear longer, softer hairs. Hind dorsal margin of 10 with a wide shallow median emargination. Grooves separating the sternites of 1 to 9 from the inflexed ventral parts of the tergites more distinct in the exuviae than on the larvae, in some of which latter they are very slightly marked.

Caudal "gills" three, evidently much subject to injury and regeneration, when fully developed each two-jointed, the first joint thicker and higher than the second; median or mid-dorsal gill (Pl. XI, figs. 9, 9*d*, 9*v*) with a mid-dorsal and a mid-ventral longitudinal carina on the first joint, the mid-dorsal carina being more convex and armed with shorter, stouter spines, while the mid-ventral carina is less convex and bears longer, more slender hairs. Each lateral gill (Pl. XI, fig. 7) with two longitudinal carinae on the mesal surface of the first joint, one of which is dorsal the other ventral; between these two carinae is thus a longitudinal trough or valley; in profile view the dorsal surface is more convex than the ventral. The second joint in all three gills is subcylindrical. When only one joint is present in any of these three gills it is probably a case of incomplete regeneration after injury. The surfaces of the three gills bear many soft hairs in addition to those on the carinae but can not be described as densely hairy.

Cercoids (superior appendages of the imago) slender, tapering, curved ventrad and applied closely for their entire length to the hind surface of abdominal segment 10.

♂. Rudiments of the copulatory apparatus visible on the ventral side of abdominal segment 2 in the larger larvae and in the exuviae. Gonapophyses on 9 represented in the larger larvae by two slightly elevated subcircular areas separated from each other by about the diameter of one of them, each bearing at its hind end 1 to 3 posteriorly-directed spines.

♀. Gonapophyses of abdominal segments 8 and 9 of variable length as indicated in the table on page 304.

The larvae preserved in alcohol have the body ochre with black markings.

Some of the internal organs of these larvae will be considered in the next number of these Studies.

TABLE SHOWING DIFFERENCES BETWEEN THE LARVAE AND EXUVIAE OF THAUMATONEURA ARRANGED ACCORDING TO SEX AND SIZE

All *measurements* in millimeters	Larva No. 1, ♂	Larva No. 2, ♂	Larva No. 9, ♂	Larva No. 5, ♂	Larva No. 4, ♂	Exuvia *inopinata* ♂	Exuvia *pellucida* ♂	Larva No. 3, ♀	Larva No. 6, ♀	Larva No. 7, ♀	Larva No. 8, ♀	Exuvia April 26, ♀
Total length..............................	14.5	17.	23.	23.5	31.	?	34.	20.5	22.	?	26.5	34.
Length excluding caudal gills.............	10.	12.	15.	16.5	21.5	26.5	27.	15.5	17.	17.5	18.	24.5
Length of abdomen excluding caudal gills	5.	6.7	7.5	10.	11.	16.	15.	8.	9.8	10.5	11.75	14.5
Width of head at the eyes,...............	3.	4.	5.25	4.	6.3	6.5	6.8	4.5	?	4.5	5.	6.1
Width of abdomen at sigment 8...........	2.5	3.3	4.	3 5	?	3.25	4.5	3.5	?	4.	4.	4.2
Length of third femur....................	2.5	3.5	4.5	3.5	5.8	5.5	6.	4.	4.2	4.3	4.75	5.9
" " tibia.......................	2.75	4.	5.5	4.	6.	6.	6.5	4.5	4.5	4.6	5.5	6.4
Width of labium at distal margin of median lobe..............................	2.	2.74	3.5	2.8	4.68	4.45	4.7	3.07	3.33	3.42	3.71	4.8
Number of crenulations on one-half of the distal margin of median labial lobe..	21	27(R),24(L)	31	24	35	34	34	29	31	30	31	36
Front wing pads reach to..................	one-half metathorax	not to hind edge metanotum	five-sixths abd. seg. 3	hind edge metanotum	?	hind edge abd. seg. 3	hind edge abd. seg. 4	one-half abd. seg. 1	hind edge abd. seg. 1	one-third abd. seg. 1	hind edge abd. seg. 2	five-sevenths abd. seg. 4
Hind " "	front edge ab. seg. 1	hind edge abd. seg. 1	five-sixths abd. seg. 4	hind edge abd. seg. 1	?	middle abd. seg. 4	hind edge abd. seg. 4	middle abd. seg. 2	hind edge abd. seg. 2	one-half abd. seg. 2	middle abd. seg. 3	two-sevenths abd. seg. 4
Length of first joint, median caudal gill....	2.	2.5	—	3.	6.	—	3.5	3.	3.5	—	3.25	3.2
" second " " "	—	1.5	—	1.5	—	—	2.5	2.	1.5	—	1.75	2.2
Total length median caudal gill...........	2.	4.	—	4.5	6.	—	6.	5.	5.	—	5.	5.4
Length of first joint, right lateral caudal gill	2.	2.	—	4.	5.	—	5.	3.	—	—	2.25	4.7
" second " " " " "	1.	1.5	—	3.	3.5	—	6.	2.	—	—	1.25	4.9
Total length " " " "	3.	3.5	—	7.	8.5	—	11.	5.	—	—	3.5	9.6
Length of first joint, left " " "	3.	3.	4.	4.	5.25	2.5	4.5	—	—	—	4.	4.3
" second " " " " "	2.	2.	4.	3.	4.75	2.	4.	—	—	—	4.	4.1
Total length " " " "	5.	5.	8.	7.	10.	4.5	8.5	—	—	—	8.	8.4
Rudiments of copulatory apparatus on abdominal segment 2, ♂	—	—	—	—	+	+	+					
Rudiments on gonapophyses on abdominal segment 9, ♂.........................	—	—	(+)	—	+	+	+					
Gonapophyses of abdominal segment 8, ♀, reach to................................								one-half abd. seg. 9	two-thirds abd. seg. 9	less than half abd. seg. 9	beyond abd. seg. 9	six-sevenths abd. seg. 10
Median gonapophyses of abdominal segment 9, ♀, reach to								three-fourths abd. seg. 9	four-fifths abd. seg. 9	three-fifths abd. seg. 9	one-half abd. seg. 10	not visible
Outer gonapophyses of abdominal segment 9, ♀, reach to.......................								hind edge abd. seg. 9	hind edge abd. seg. 9	six-sevenths abd. seg. 9	three-fifths abd. seg. 10	six-sevenths abd. seg. 10

Explanation of Plate XI.

All the figures are of *Thaumatoneura* larvae or of parts thereof.

Figs. 1 to 3 are from photographs by Mr. H. A. Walters, of alcoholic specimens.

Fig. 1, larva No. 8, ♀, dorsal view. x 2.

Figs. 2, 3, larva No. 4, ♂, dorsal and ventral views respectively x 1.8. The median caudal gill is detached and lying on its side.

Figs. 4 to 9 are from camera drawings made with a binocular stereoscopic, or a compound, microscope; figs. 4 to 6 and 8 are from larva No. 4, ♂.

Figs. 4, 5, ventral views of the right and left mandibles respectively of larva No. 4, ♂. *d, b, c, v,* the apical teeth, *d* the most dorsal, *v* the most ventral; d^1 the most dorsal, v^1 the most ventral, spine of the inner process of the left mandible; *abt, adt,* tendons of the abductor and adductor muscles respectively. x 12.

Fig. 4*i*, curved inner process of the right mandible viewed from the base of the mandible to show the minute ante-apical denticle.

Fig. 5*i*, outline of the end view, or mesal surface, of the inner process of the left mandible.

Fig. 6. Labium, dorsal surface. *abl* abductor, *adl* adductor of the lateral lobe; *flm* flexor of the mentum, *flt* flexor of the terminal or movable hook. x 5.

Fig. 7. External view of left lateral gill of larva No. 5, ♂, drawn *in situ* on the larva. x 5.

Fig. 8. Right half of the distal margin of the median labial lobe, t^* tooth. x 53.

Fig. 9. Left profile view of the median caudal gill of larva No. 6; 9*d* a small piece of the dorsal edge of proximal joint of the same gill; 9*v* a small piece of the ventral edge of proximal joint of the same gill. Fig. 9, x 5, 9*d* and 9*v*, x 30.

Studies on Costa Rican Odonata.

VII. The Waterfall-Dwellers: The Internal Organs of Thaumatoneura larva and the Respiration and Rectal Tracheation of Zygopterous Larvae in general.

By PHILIP P. CALVERT, PH.D., University of Pennsylvania, Philadelphia, Pa.

(Plates XV, XVI, XVII, fig. 1)

The caudal tracheal gills of the larva of *Thaumatoneura*—to give the three most conspicuous, terminal, abdominal parts the name which they have received in other Zygopterous larvae—have been described and figured in No. VI of these Studies.* They are very clearly two-jointed, a condition which has also been described and figured by Needham† for the same parts of the larvae of *Bayadera indica,* of an unknown genus and species from Jamaica, of an unknown genus of the legion Podagrion from India and of a species of *Pseudagrion.* The resemblance between his figure (*l. c.,* pl. v, f. 4) of a lateral caudal gill of *Bayadera indica* and ours (Ent. News, xxvi, pl. xi, f. 9) of a median caudal gill of *Thaumatoneura* is very close. This two-jointed condition is of interest since these gills are homologous to the many-jointed middle tail filament and cerci of *Lepisma* and of Ephemerid larvae, while the lateral gills are homologous to the several- to many-jointed cerci of Orthoptera and of larval Perlidae (Heymons 1896, 1904). Heymons termed the median unpaired "gill" the *appendix dorsalis* and the other two the *appendices laterales,* the three collectively *appendices caudales,* applying these terms both to Zygopterous and Anisopterous larvae.

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*Ent. News, xxvi, pp. 295-305, pl. xi. July, 1915.

†Ent. News, xxii, pp. 145-154, pls. iv, v; pp. 342-347, pl. xi. 1911.

The three caudal appendages of *Thaumatoneura* larvae, although supplied with tracheae whose derivation is homologous with that of the tracheae of the lamellate caudal gills of most Agrionine larvae, present so little surface for respiration that I expected dissection to reveal some rectal tracheal gills comparable with those of the larvae of the Anisoptera. This expectation has been partly fulfilled but, as will be seen from the present paper, the rectal folds in *Thaumatoneura* larva are less numerous and each of the three folds less sub-divided and much less tracheated than is the case in Anisopterous larvae. No observations or experiments bearing directly on the methods of respiration were made on the living larvae of *Thaumatoneura.* We know that in their native waterfalls they are frequently found on rock-faces where they are kept moist, but are not submerged, by the falling water. Their movements, both at large and in our rearing glasses, were never rapid.

INTERNAL ORGANS OF THAUMATONEURA LARVA.*

Alimentary canal. The *pharynx* is separated from the oesophagus by a distinct constriction lying a little caudad to the level of the brain, but cephalad to the level of the hind margin of the head; the *oesophagus* is defined posteriorly by a constriction in the prothorax separating it from the crop (larva no. 6). The external dorsal surface of the oe-

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*Owing to the destruction referred to under the heading "Description of the Larvae," Ent. News, xxvi, p. 300, no larvae specially fixed for histological study have been available, and it has been necessary to rely exclusively on larvae nos. 1-9 (*l. c.*), which were placed in alcohol as soon as their deaths were discovered.

sophagus is marked by two longitudinal streaks of blackish pigment corresponding to the areas of contact with the right and left dorsal tracheal trunks. At mid-length of the oesophagus, at the level of the hind margin of the head, is, on each lateral surface, right and left, a *salivary gland* with a delicate duct running cephalad. The *gizzard* lies in the first abdominal segment; its chitinous armature, in larva no. 4, consists of sixteen longitudinal folds, each of which bears chitinous teeth. In eight of these folds the teeth, near the posterior end, tend to increase from one to two rows, while in the other alternating eight folds the teeth are arranged in a single row throughout. The teeth of the various folds are subequal in size, but in each fold tend to become larger caudad. The formula for the gizzard armature may be written 8 (F 14-17, f 11-14)* The hind end of the *stomach* and the place of attachment of the *Malpighian tubes* lie near the articulation of the fifth and sixth abdominal segments. The *ileum* extends to the seventh segment.

The *rectal epithelium* presents three longitudinal folds, one which is dorsal and a little to the left of the median plane, a second is left latero-ventral, the third is right lateral. Their relative positions may be best seen in a cross-section of the rectum (Pl. XVII, fig. 1). The epithelium is interrupted by three, longitudinal, thinner, pigmented areas, one of which is nearlv midventral (*mvpa*) and lies immediately to the right of the attachment of the left latero-ventral fold, a second (*rlpa*) is immediately dorsal to the right lateral fold, the third (*llpa*) is to the left of the dorsal fold. These three pigmented areas are homologous with the three "non-cellular longitudinal bands" of the rectum of the larva of *Mecistogaster modestus*† and with similarly placed bands in the recta of larvae of *Lestes rectangularis, Argia moesta putrida, A. talamanca, Ischnura verticalis,* etc. Both anterior and posterior to the three longitudinal rectal folds in *Thaumatoneura* larva, the gut epithelium is very much thinner for its entire circumference, equaling, or nearly equaling, in thinness that of the three longitudinal pigmented areas, and containing also much pigment.

Tracheal System. The arrangement of the larger tracheae of the dorsal side of the body is shown in Plate XV. It corresponds, except in a few minor details, with our figure** for the tracheae of *Mecistogaster modestus* larva and those of Cole†† and of Bervoets‡ for a larva of *"Agrion."* The chief difference between the present results and those of the Belgian author is that our serial sections through the posterior part of the abdomen do not show a pair of independent "ventral trunks" which he partially figures and describes.

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*Cf. Higgins, Proc. Acad. Nat. Sci. Phila. 1901, pp. 132, 133.

†Ent. News, xxii, pp. 452, 453, pl. xvii, fig. 10, 1910. The description of them as "non-cellular" may not be justified when material in better condition is examined.

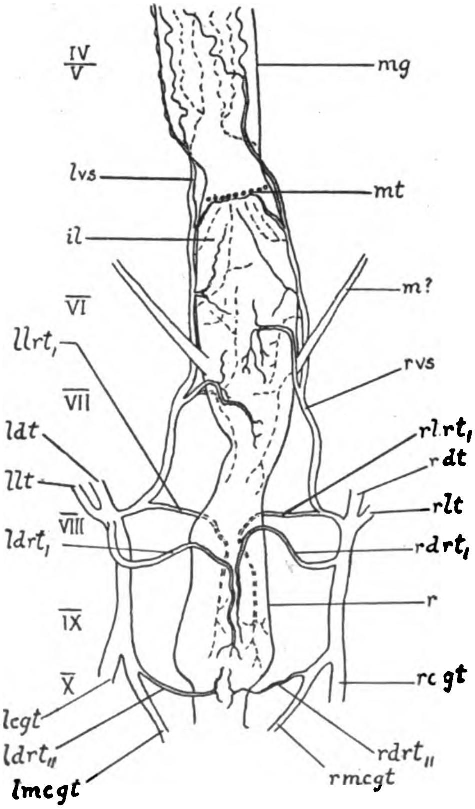
**Ent. News, xxii, pl. xvii, fig. 8. 1911.

††Journ. Appl. Micros. & Laby. Meth. VI, pp. 2224-5, figs. 7, 8. 1903.

‡Annales Biol. Lacustre, VI, pp. 16, 17, 20, figs. 1-3. 1913.

The *tracheae which supply the rectum* have been studied by a series of transverse serial sections through the hind part of the abdomen of larvae nos. 2 and 7. In spite of the unfavorable condition of the material for a histological investigation, the tracheae were found to be intact even to branches of small diameter (.002 mm.). The rectal epithelium being in a much better state of preservation in larva no. 7, the following brief description and the figures on Plates XVI and XVII have been based chiefly upon that larva.

The two great *dorsal longitudinal tracheae*, right and left, *rdt*, *ldt*, each give off, in the anterior part of the eighth abdominal segment, a



Text-figure 1. The principal tracheae supplying the alimentary canal in the posterior half of the abdomen, *Thaumatoneura* larva, ♂, No. 4. Dorsal view. x 18.8 For explanation of the abbreviations, see page 394.

branch, the *dorsal rectal trachea,* (*rdrt*1, *ldrt*1), which runs mesad and enters the cavity of the dorsal longitudinal rectal fold. Within this fold this trachea divides and subdivides, but no anastomosis between any of the smallest subdivisions of the right dorsal rectal trachea with subdivisions of the left dorsal rectal trachea have been found, in spite of special search for them.

A short distance anterior to the origin of each dorsal rectal trachea a *lateral rectal trachea* (*rlrt*1, *llrt*1), arises, either indirectly from the dorsal longitudinal trachea (*cf.* text-figure 1 and right side of Plate XVI), or from the lateral longitudinal trachea (*cf.* left side of the same Plate), which extends mesad and caudad and enters the lateral longitudinal rectal fold of the same side of the viscus, within which it subdivides. A smaller, posterior, tracheal supply (*rlrt*11, *llrt*11) is also received by these lateral rectal folds from branches either from the lateral longitudinal trachea or from the median caudal gill trachea, as may be seen by comparing the right and left sides respectively of Plate XVI. No anastomoses of tracheoles from the anterior supply of the lateral rectal folds with those of the posterior supply have been detected.

A small posterior tracheal supply for the dorsal rectal fold is received from the right and left median caudal gill tracheae (tracheae *rdrt*11, *lrdt*11 of Plate XVI). Here again no anastomoses, either transverse or longitudinal, have been found.

The only anastomosing tracheae which have been discovered are the posterior terminations of the right and left lateral longitudinal tracheae which, assuming a ventral position, meet on the mid-ventral line ventrad of the alimentary canal; in this anastomosis (*anas*) take part also a branch from the right and a branch from the left lateral caudal gill trachea. These four anastomosing tracheae, since they lie ventrad to the rectum, have been represented by lines of alternating dots and dashes in Plate XVI. A similar anastomosis in *Mecistogaster modestus* has been figured* in No. III of these studies and has been found by the writer in transparent living larvae of *Hetaerina americana, Argia moesta putrida* and *Ischnura verticalis;* it has not been figured for Anisopterous larvae and may prove to be a characteristic of those of the Zygoptera.

A comparison of the works of authors who have described the tracheae of Odonate larvae has yielded the table on page 390.

The *terminal tracheoles,* of the tracheae which run into the longitudinal rectal folds, enter the epithelium, although whether they merely pass between the epithelial cells or actually penetrate the latter, the condition of the material does not enable one to decide. The diameter of some of these infra-epithelial tracheoles, near their point of final

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*Ent. News, xxii, pl. xvii, fig. 7. 1911.

HOMOLOGIES OF THE PRINCIPAL LONGITUDINAL TRACHEAE OF ODONATE LARVAE AS DESCRIBED AND FIGURED BY DIFFERENT AUTHORS.

Author, date, reference and species		Names of Tracheae adopted in this paper.		
		Dorsal	Visceral	Lateral
Carus, 1827, Taf. II, fig. 2 and p. 15 [*Lestes sponsa*]		*d* [on left side of figure only] zwei zu beiden Seiten des Leibes verlaufende Tracheenstämme......den grossen Tracheenstämmen.		
Suckow, 1828. Taf. I, figs. 7, 9. *Aeshna grandis*		*o*, *o* zwei grosse Tracheenstämme	*q* ein geradelaufender	*p* ein dünnerer schlangenförmiger Tracheenstamm
Dufour, 1852. Pl. 5 and pp. 77-79	Fig. 28 *Aeshna grandis*	*hh*, canaux aérifères supérieures	*ii* canaux aérifères intermédiaires	Canaux aérifères inférieures (pp. 78. 79)
	Fig. 32 *Calopterix virgo*	*i* canal aérifère supérieure		
	Fig. 33 *Agrion puella*	*hh*, canaux aérifères supérieures		Canaux aérifères inférieures (pp. 78, 79)
Oustalet, 1869. Pl. 22, fig. 1. *Aeschna maculatissima*		*A*, *A* troncs supérieurs ou *dorsaux*	*BB*, troncs moyens ou *visceraux*	*CC*, troncs inférieurs ou *ventraux*
Palmén, 1877. Taf. I, fig 14 and p. 37. *Agrion.*		*trl* die beiden dorsal	Der dritte Längsstamm	*trl* *1* secundären Längsstamm, den ventralen
Roster, 1885. Tav. III and pp. 260-261 *Aeschna cyanea.*		paio esterno* e dorsale; canali centrali superiori	paio viscerale; canali viscerali; tronchi viscerali	paio ventrale o laterale; tronchi ventrali o esterni*
Scott, 1905. Figs. 1, 2 and pp. 345-349 *Plathemis lydia.*		*DORS* The dorsals	*VENT* The ventrals	*LAT* Lateral system
Calvert, 1911*a*. Pl. III, fig. 22. *Cora.*		*ldt*, *rdt*, dorsal tracheae	*lpgt*, (left) posterior gastric trachea	*llt*, *rlt*, lateral tracheae
Calvert, 1911*b*. Pl. XVII, fig. 8. *Mecistogaster modestus.*		*ldt*, *rdt*, dorsal tracheae		*llt*, (left) lateral trachea
Bervoets, 1913. Figs. 1, 2. *Agrion.*		gros troncs lateraux	troncs ventraux	troncs lateraux ventraux †

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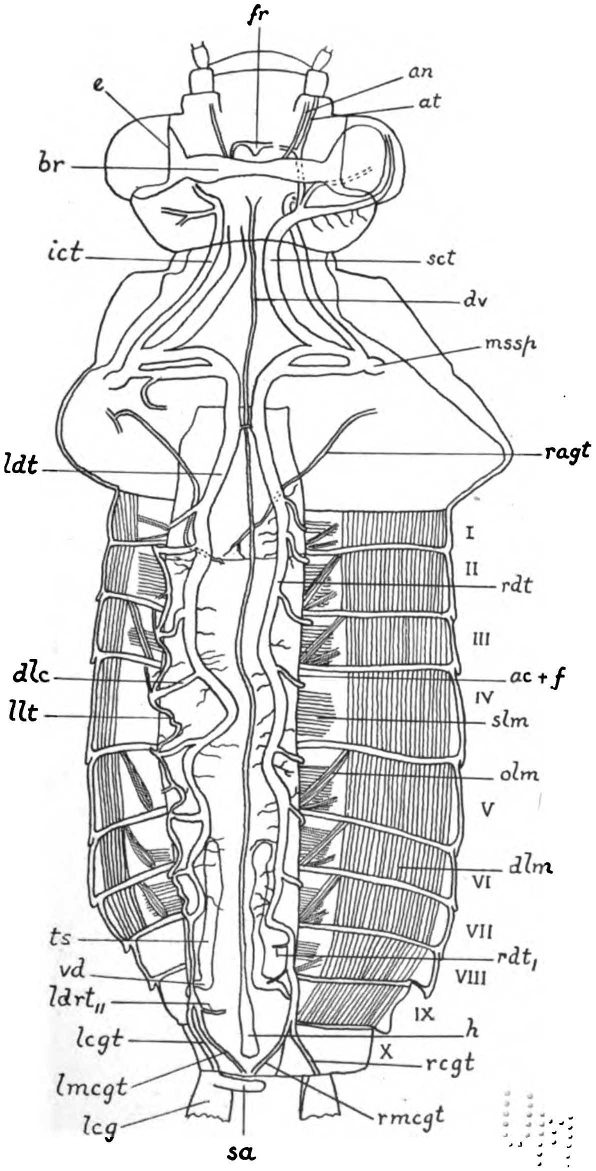
* Roster has applied the terms *esterno*, *esterni*, to both of these tracheae.

† Bervoets adds (p. 18), "Cette pair de troncs .. pourrait être appelée viscérale. Chez les Anisoptères, ces troncs se croisent sur le ventricule chylifique." I think, however, that he is in error in this homologization.



THAUMATONEURA LARVA—CALVERT.

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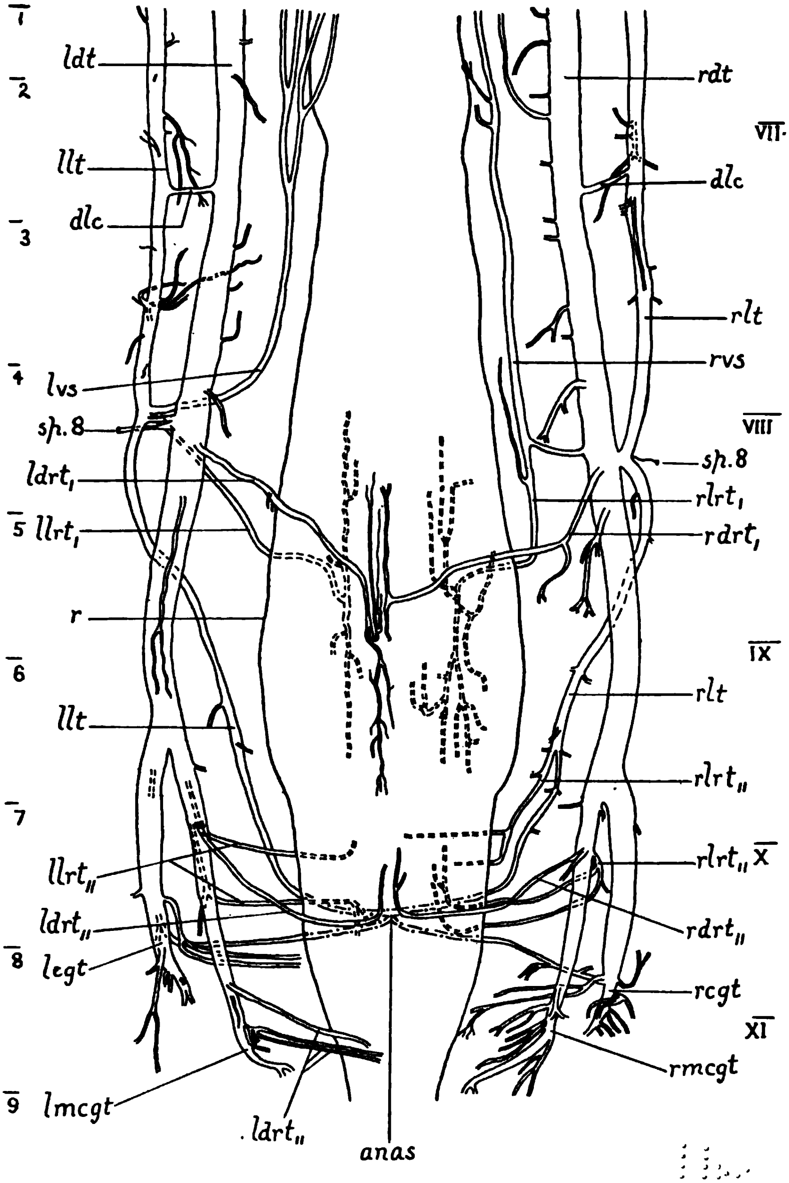
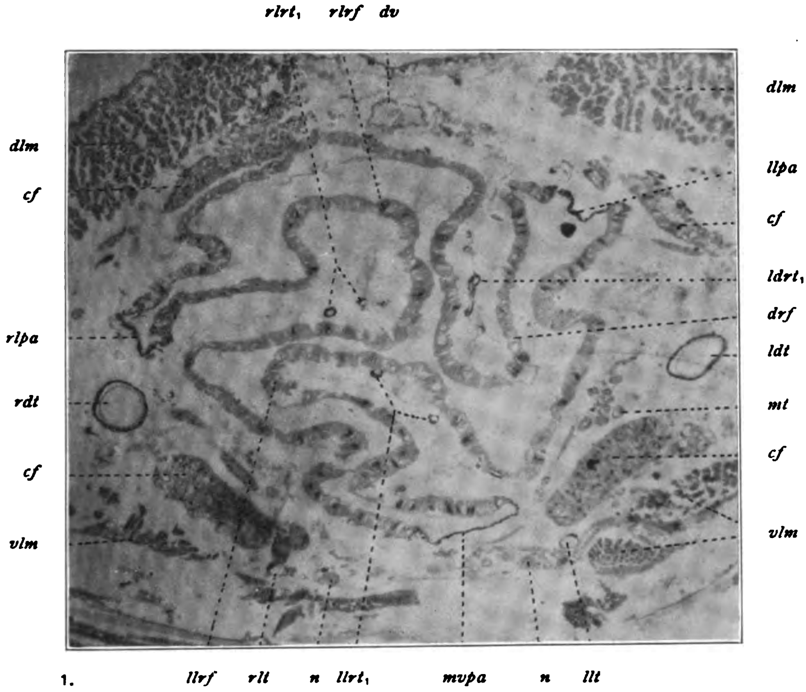


THAUMATONEURA LARVA—CALVERT.



1. THAUMATONEURA LARVA—CALVERT.

disappearance in the serial sections, is but .002 mm.* The manner of termination has not been ascertained.

The great majority of the tracheae in the abdomen, of all sizes from the great longitudinal trunks to the infra-epithelial tracheoles, possess an abundance of *blackish pigment* in their own epithelial layers. This pigment is evident both in entire preparations and dissections mounted in balsam and also in the sections from blocks of material embedded in paraffin, and greatly increases the ease with which these vessels may be traced. It presents the same appearance as that of the pigment in the "three, longitudinal, thinner pigmented areas" of the rectum, described above, and in other parts of the body, such as the epithelial layer of the pharynx, the connective tissue envelopes of the frontal ganglion, of the brain and of at least some of the ganglia of the ventral nerve cord.

This pigment is perhaps the same as that for which Purser (1915, p. 67) has proposed the name "spadicin" and which he suggests may have a respiratory function. His statements that spadicin "only appears in the respiratory organs of true aquatic insects" (p. 68) and "is not situated in the tracheal epithelium but in the hypodermis" [of Agrionid larval tracheal gills and in those of *Aeschna?*] (p. 69) are not entirely in accord with conditions here described for *Thaumatoneura.*

Transverse sections of the hind end of the abdomen and of *median and lateral caudal gills* show that the tracheae, supplying the latter organs do not enter them as single, relatively large trunks, but that each trachea divides and redivides into a number (four or more) of subequal tracheae which collectively pass into the base of the gill. Neither the sections of the gills nor entire mounts of these organs indicate any rich development of tracheae within them. The thickness of the chitinous cuticle of the proximal joint, as measured in cross-sections of the median caudal gill and of one lateral caudal gill of larva no. 2, varies from .012 to .016 mm., and .004 to .016 mm. respectively. There is much less blackish pigment in the epithelial layer of the tracheae of these gills than in that of the tracheae of the abdomen. Pigment is present in the epithelium of the gills themselves, but is not uniformly distributed therein.

The same sections of the median caudal gill show the presence of two median *blood spaces,* one dorso-central, the other ventro-central, situated respectively ventrad and dorsad to other blood spaces which largely occupy the mid-dorsal and mid-ventral longitudinal carinae of the proximal joint. These two central blood spaces have thin but distinct walls, contain plasma and corpuscles in their lumens and lie in the midst of a reticulated or spongy tissue; their transverse diameter, near

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***E. g.,* in the dorsal fold, slide 5, row 3, section 20, of our series of larva no. 7.

the base of the gill, is about one-fifth of that of the gill itself. More posteriorly they are smaller, both relatively and absolutely, and they are not shut off from communication with other spaces. In the caudal end of the proximal joint, the two central blood spaces become confluent as one central space, whose vertical diameter is almost as great as that of the gill itself exclusive of the mid-dorsal and mid-ventral carinae. This central blood-space is continued caudad into the distal joint, occupying most of the interior thereof. The chitinous cuticle of this joint is, in these sections, .012 mm. thick.

The lateral caudal gill has a dorsal and a ventral longitudinal blood-space near the two longitudinal carinae of the mesal surface, respectively. A third appears to run along its lateral or external face.

A number of resemblances between the internal structure of the caudal gills of *Thaumatoneura* and those of *Euphaea variegata* described by Ris (1912, pp. 171-3) are evident.

Nerve ganglia. The positions of the ganglia other than the brain are as follows. The *frontal* ganglion, triangular in shape, with its apex directed caudad (Pl. XV, *fr*), lies on the dorsal surface of the pharynx a short distance (about .15 mm. in larva no. 2) anterior to the brain; it is connected on each side, right and left, with the cerebro-suboesophageal connective. *Suboesophageal* ventral to the pharynx, at the level of mid-length of the eyes. *Prothoracic* in the hind end of the prothorax, *mesothoracic* in the front part of the mesothorax, *metathoracic* in the hind part of the mesothorax; the thoracic ganglia are separated from each other by intervals shorter than the length of each. *First abdominal* in the hind part of the metathorax, *second* in the middle of abdominal segment 2, *third to seventh* in the front ends of segments 4 to 8 respectively. These positions of the thoracic ganglia are obtained from larvae nos. 2, 4, and 6, of the abdominal from larvae nos. 4 and 6.

(To be continued)

EXPLANATION OF PLATES XV, XVI, XVII.

PLATE XV. Dorsal tracheae and some other organs of *Thaumatoneura* larva ♂, no. 4. The dorsal wall of the head has been removed except over the eyes, that of the thorax and of the abdomen has been opened longitudinally to the left of the mid-dorsal line and turned to the right. x 6.1.

PLATE XVI. Diagram of the tracheal supply of the alimentary canal in the hind part of the abdomen of *Thaumatoneura* larva, dorsal view. x 37.6.

This diagram has been reconstructed on a horizontal plane from about 850 serial transverse sections from larva no. 7, mounted on 9 slides now in the writer's collection at the University of Pennsylvania. To avoid confusing tracheae which lie approximately in the same vertical planes, the tracheae are represented farther to right and to left from the gut and from each other than is actually the case. The true relative positions may be learned by comparing this figure with figure 1 of Plate XVII. The origins of the various branch-tracheae and their subdivisions to the most minute branches have been indicated as exactly as possible from the sections. The tracheae which do not lie upon the gut wall or do not enter the rectal folds have not been shown farther than short distances from their origins, even though they may lie dorsad or ventrad to the gut; this for the sake of avoiding confusion. The only exceptions to this statement are in the cases of the tracheae which take part in the anastomosis (*anas*) mid-ventral to the rectum in abdominal segment X; these tracheae are indicated by lines of dashes alternating with dots, thus — . — . — . — . Of the tracheae which supply the rectal walls, those which run on the dorsal surface or enter the dorsal longitudinal fold are shown in solid lines; those which run on the ventral surface or enter the lateral rectal folds are shown in broken lines, thus — — —, as soon as they cross the outlines of the gut.

In one respect this diagram departs from the conditions shown in the sections of larva no. 7. In those sections the right lateral trachea (*rlt*) becomes much smaller in abdominal segment VII than its fellow of the left side and disappears as several minute tracheae before connecting again with the dorsal trachea. This, after comparison with other larvae, appears to be an anomaly, so that the right lateral trachea is here represented as being similar to, but not identical with, the left lateral. The branch tracheae *rvs, rlrt*1, which on the right side of the diagram come from the dorsal longitudinal trachea, are exactly as in the sections, although the corresponding tracheae *lvs, llrt*1, on the left side come from the lateral longitudinal trachea. This difference in the two sides of larva no. 7 may be due to the atrophy of the right lateral longitudinal trachea just mentioned. Compare, however, the same tracheae in text-figure 1, page 388. The right lateral trachea *is present* in the sections from its point of separation from the right dorsal in the anterior part of segment VIII as represented in the diagram.

The figures 1-9 on the left side of this plate indicate the levels of the first section of each slide, 1-9 respectively.

PLATE XVII, fig. 1. Part of photograph of section 18, row 1, slide 6, of the author's series of sections of *Thaumatoneura* larva no. 7 ♀, made with Leitz oc. 4, obj. 3, by Mr. H. A. Walters. The section is viewed from its anterior surface. x 72.5.

Abbreviations Used in Plates XV-XVII.

ac + *f*, outline of alimentary canal and superimposed fat masses; in Plate XV it corresponds also very nearly to the lateral edges of the sternites.

an, antennal nerve.

anas, anastomosis of tracheae in abdominal segment X.

at, antennal trachea.

br, brain.
cf, connective tissue and fat.
dlc, transverse tracheae connecting the dorsal and lateral longitudinal tracheae.
dlm, dorsal longitudinal muscles.
drf, dorsal rectal fold.
dv, dorsal vessel.
e, eye, mesal outline.
fr, frontal ganglion.
h, heart.
ict, inferior cephalic trachea (to ventral muscles of head).
il, ileum.
lcg, left caudal gill.
lcgt, left caudal gill trachea.
*ldrt*I, anterior or chief left dorsal rectal trachea.
*ldrt*II, posterior or secondary left dorsal rectal trachea.
ldt, left dorsal trachea.
llpa, left lateral pigmented area of rectum.
llrf, left lateral rectal fold.
*llrt*I, anterior or chief left lateral rectal trachea.
*llrt*II, posterior or secondary left lateral rectal tracheae.
llt, left lateral trachea.
lmcgt, left median caudal gill trachea.
lvs, left visceral trachea.
m, muscle.
mg, midgut (stomach).
mssp, mesostigma (mesothoracic spiracle).
mt, Malpighian tubes.
mvpa, midventral pigmented area of rectum.
n, nerves (in Pl. XVII, fig. I, running caudad from last ganglion)
olm, oblique lateral muscle.
r, rectum.
ragt, right anterior gastric trachea.
rcgt, right caudal gill trachea.
*rdrt*I, anterior or chief right dorsal rectal trachea.
*rdrt*II, posterior or secondary right dorsal rectal trachea.
rdt, right dorsal trachea.
rlpa, right lateral pigmented area of rectum.
rlrf, right lateral rectal fold.
*rlrt*I, anterior or chief right lateral rectal trachea.
*rlrt*II, posterior or secondary right lateral rectal tracheae.
rlt, right lateral trachea.
rmcgt, right median caudal gill trachea.
rvs, right visceral trachea.
sa, superior appendages of imago ("cercoids").
sct, superior cephalic trachea (to antenna, brain, optic lobe and eye).

slm, straight lateral muscle.
sp.8, site of future spiracle of 8th abdominal segment.
ts, testis.
vd, vas deferens.
vlm, ventral longitudinal muscles.

The Roman numerals I-XI indicate abdominal segments 1-11 respectively.

The names of the abdominal muscles are, as nearly as possible, those employed by Matsula in Pflüger's Archiv f. Physiologie, cxxxviii, pp. 390-392, text fig. 1, 2. 1911.

Studies on Costa Rican Odonata.

VII. The Waterfall-Dwellers: The Internal Organs of Thaumatoneura larva and the Respiration and Rectal Tracheation of Zygopterous Larvae in general.

(Continued from page 395)

By PHILIP P. CALVERT, PH.D., University of Pennsylvania, Philadelphia.

Our knowledge of the whole subject of the respiration of Zygopterous larvae is still very indefinite, and it seems worth while to summarize it such as it is.

CLASSIFICATION OF THE TRACHEAL GILLS OF ODONATE LARVAE.

Dufour (1852), grouping all the Odonate larvae known to him under the four genera, *Aeshna, Libellula, Calopterix* and *Agrion,* gave as generic characters for the first three "Branchies intérieures ou rectales," and for the last "Branchies extérieures ou *caudales* . . . et servant aussi de *nageoires.*" He added "Comme on voit, la nature et la position des branchies établissent entre le *Calopterix* et *l'Agrion* une distance anatomique énorme" (p. 72). Hagen (1853), recognizing five families, gave for Libelluliden, Gomphiden and Aeschniden "Darmkiemen," for Calopterygiden "Darm und Schwanzkiemen," for Agrioniden "Schwanzkiemen" (pp. 261-9, 311). Rousseau (1909, pp. 2, 43, 45) has accepted the same distinctions so far as "branchies" are concerned.

Roster (1885, p. 259), on the other hand, grouped *Agrion, Lestes* and *Calopteryx* as Caudobranchiati, *Anax, Libellula* and *Aeschna* as Rectobranchiati, although Dufour (1852, p. 83) had employed the same words, in the form of French adjectives, in the different sense implied by his classification quoted above. Roster's terms were synonymous, as group names, with the earlier Zygoptera and Anisoptera and were so recognized by systematic writers.*

FUNCTIONS OF THE THREE CAUDAL APPENDAGES OF ZYGOPTEROUS LARVAE.

Réaumur (1742, p. 405), who regarded these appendages as "nageoires" (fins), and Roesel von Rosenhof (1749, pp. 41 et seq.), who termed them "Ruder-Federn" (rudder feathers), say nothing of any respiratory function for them. Without attempting to determine who was the first author to definitely describe them as gills, it is worth recalling that it was in them that Carus (1827) first discovered the circulation of the blood in insects. To these organs he applied the terms "Schwanzblättchen," "kiemenartigen Blättchen" and "Schwanzkiemenblättchen" (pp. 9, 14), and he figured one of them on a large scale (Taf. I, f. 4). He termed the species which he studied *Agrion puella* and although the specific identification is probably not correct, it is evident that he had before him a typical Agrionine.*

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*E. g., de Selys-Longchamps. Ann. Soc. Ent. Belg. xxxi, pp. 75, 80, in a "Liste des Odonates d'Europe en 1887."

By Dufour (1852, as already quoted), and by Roster (1886, p. 242), the two functions of respiration and of locomotion were accepted without question. Roster went so far as to conclude that

> a branchial lamella is reproduced when the individual has reached such [a state] that it is not able to accomplish its development without these necessary organs and that the presence of one lamella, although compelling the insect to a forced rest, suffices to satisfy all the respiratory needs of the organism. The great variability in the dimensions of these insects can find a cause in the defective development to which a physical imperfection, such as the absence of two respiratory lamellae, gives rise (p. 245).

The observation that Agrionine larvae can live without their caudal appendages dates back at least to Roesel (1749, p. 50). Hagen (1853, p. 311), Dewitz (1890, p. 504), and Janda (1910*a*, p. 32, 1910*b*, p. 607) inferred from this survival some sort of rectal respiration.

Sharp (1895, p. 422) thought that the respiratory function of the caudal appendages "must be of an accessory nature,

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*It may be mentioned here that the second species which Carus studied and figured as "eine kleine Neuropteren-Larvae," "vielleicht zu *Semblis, Sialis,* od. dergl. gehörig," was also a Zygopterous larva, as von Siebold pointed out (Archiv f. Naturgesch, VII, 1 Bd., p. 211, 1841) that it resembles the larvae of *Agrion forcipula* [= *Lestes sponsa* Hansem.] Packard's implication (1898, p. 397) that it was in the larva of *Ephemera* that Carus made his *first* observation of circulating blood is incorrect.

for the nymphs live after the removal of the processes . . . [and] the skin of these processes is harder than is usual in Insect gills." Similar views were also expressed by Heymons (1904, pp. 23, 24):

> The exchange of gas during this period [youngest larval stages] is accomplished without doubt not solely through the appendages, but in a sufficient way through the entire body-surface which at first is covered with extremely delicate chitin....During the larval period there can be no doubt that the three appendages have become primarily breathing organs from the physiological standpoint. But, as earlier, the entire body-surface serves for respiration in addition to the gill-appendages and a loss of the appendages in no way leads to death of the animal.

Tillyard (1909, pp. 381-2), discussing the caudal appendages of the larva of the Australian *Diphlebia,* while admitting that "the presence of numerous branched tracheae in these gills" must mean "that *originally* they were in some way organs of respiration," points out that the loss of these gills "does not affect the respiration of the insect in any way," and doubts "whether even the wide lateral gills of *Diphlebia,* fed by two large tracheae, are of any use at present for auxiliary respiration."

RECTAL RESPIRATION. CALOPTERYGINAE.

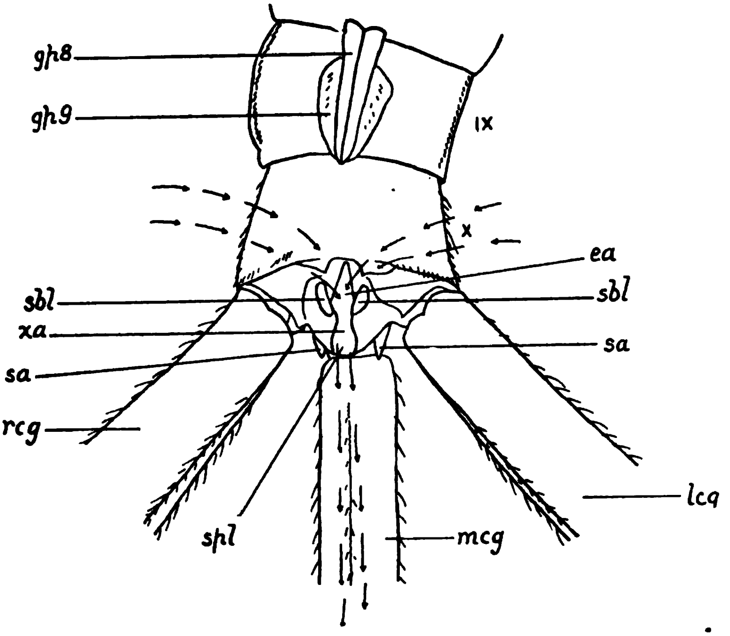
The earliest record of observations on rectal respiration in this group appears to be that of Roesel von Rosenhof (1749, p. 43) when he says of the nymphs [of *Calopteryx*] that they draw "their air not through the mouth, but through the abdomen, and such can easily be perceived in them if they are kept in a clear glass full of water." Dufour (1852, p. 91) saw larvae of *Calopterix* "swallow through the anus the water which serves for their respiration." He described (1849, 1852, p. 87) gills in the rectum as forming three membranous folds ("raquettes") fixed by a single extremity to the inner and posterior part of the stercoral pocket in such manner as to be free and floating for two-thirds of their length. Hagen (1880*a*, p. 160) found these gills "genau wie Léon Dufour." This statement was called forth by Olga Poletaïew's denial (1880, p. 450) of the existence of rectal gills in the larva of *Calopteryx* based on an examination of the interior envelope of the rectum in exuviae. Very recently Ris (1913, p. 95) has stated that he is disposed to consider the "older statements of Dufour and Hagen on the union in *Calopteryx* larvae of the zygopterous with a rudiment of the anisopterous organ as an error of observation."

The present writer has recently made some observations on living larvae of *Calopteryx maculata* from the neighborhood of Philadelphia, as follows:

A male larva (body-length, exclusive of caudal appendages and of antennae, 17.5 mm., median caudal appendage 6 mm., lateral caudal appendage 8.5 mm.) placed in a Syracuse watch glass at 3.25 p. m. in barely enough water to cover the larva. The larva remained very quiet and was watched under a Zeiss binocular microscope with objectives a° and oculars 2. Anal expirations at the rate of 38 per minute were indicated by movements of sediment attached to the hairs on the median caudal gill.

The same larva was turned on its back at 3.30 p. m., all the other conditions remaining the same as before. The larva remained very quiet; 36 anal expirations per minute indicated as before. Powdered carmine was sprinkled on the water and floating on its surface showed very clearly the expulsions of water from the anus at a rate of 33 per minute. The carmine particles showed currents as indicated in the accompanying diagram (Text-fig. 2). The expulsions from the anus were accompanied by alternate contractions and expansions of abdominal segments 3-8, which were most marked near the lateral edges of the segments at their articulation with the next following segment. Observations on the direction of movement of carmine particles and particles of sediment *in* (not on the surface of) the water made it seem likely that the water entered the rectum through an aperture (*ea*) between the ventral margins of the right and left subanal plates and the posterior ventral margin of abdominal segment 10, and that it left the rectum by an orifice (*xa*), less easily seen in ventral view, between the supra-anal plate and the dorsal margins of the right and left subanal plates. The chief evidence for the entrance orifice, which was not closed, was the behavior of a mass of flocculent substance, a little larger than the aperture, which, when placed over it, was rhythmically sucked inward.

A second, female, larva (body length, excl., etc., 20.5 mm., median caudal appendage 7 mm., lateral appendages 8.5 and 9 mm.) was placed on its back under the same conditions and similarly examined, carmine particles being sprinkled on the water; 40 anal expulsions per minute. In this case, however,



Text-figure 2. Ventral view of hind end of the abdomen of a female larva of *Calopteryx maculata* Beauv. From specimen 12 mm. long (excl. antennae and caudal gills) preserved in alcohol. The arrows show the direction taken by currents of water, as indicated by carmine particles suspended in the water, under the conditions described in the text, page 438. x 24.

ea, aperture between the subanal laminae, *sbl*, *sbl*, and the hind margin of segment 10; *gp8*, gonapophyses (ovipositor) of the 8th abdominal segment; *gp9*, gonapophyses (genital valves) of the 9th abdominal segment; *lcg*, left caudal gill; *mcg*, median caudal gill; *rcg*, right caudal gill; *sa*, superior appendages of imago (cercoids); *sbl*, subanal lamina; *spl*, supra-anal lamina; *xa*, aperture between the supra-anal lamina and the subanal laminae. In this figure the apertures *ea* and *xa* are shown as continuous, as they are in the preserved specimen from which the drawing was made. In the living larvae described in the text, these two apertures were separated from each other by the approximation of the subanal laminae, but internally each communicates with the rectal cavity. IX, X, ninth and tenth abdominal segments.

both entrance and exit of the water into and from the rectum seemed to be by the aperture *ea* between the ventral margins of the subanal plates and the hind ventral margin of abdominal segment 10.

In both larvae the expulsions were much more evident than the inhalations, the latter causing very much less movement of suspended or floating particles. From time to time, for intervals of one minute or longer, both inhalations and exhalations ceased.

In a transparent living larva of *Hetaerina americana* (dimensions corresponding to those of *Calopteryx* larva quoted: 8.5, 3, 4 mm.), examined under a Zeiss compound microscope, objective A, ocular 2, the rectal tracheal supply was clearly seen to be very similar to that figured for *Thaumatoneura* (Pl. XVI) in the present paper. Rhythmic pulsations in the rectum were distinctly visible at a rate of 45-53 "sets" per minute, each "set" consisting of 3-4 successive contractions of the rectal wall, followed by a longer pause of varying duration. Currents of water corresponding to the rectal pulsations were demonstrated by the use of carmine particles as described for *Calopteryx*. Water was expelled caudad from the vicinity of the anus 45 times per minute jerkwise, each jerk apparently synchronous with the pause following each "set" of rectal contractions. The ileum moved rhythmically, cephalad from the sixth to the fifth abdominal segment while the rectum was contracting, and caudad into the sixth segment again synchronously with the pause following each "set" of rectal contractions.

In the larva of *Cora*, the condition of the material did not permit of an exact statement as to the existence of rectal tracheal gills, but it was noted that the rectal walls appeared much less richly tracheated than those of the stomach. (Calvert, 1911*a*, p. 55; *cf*. pl. ii, ff. 17, 19).

RECTAL RESPIRATION. AGRIONINAE.

Schmidt-Schwedt (1891, p. 104) stated that larvae of *Agrion* and of *Lestes* do not possess respiratory movements through the anus and Tümpel (1908, p. 66) asserted that all other Agrionidae [than *Calopteryx*] lack gut-gills (Darmkiemen), but breathe only by the caudal gills. On the other hand, Dewitz (1890, p. 504) saw in transparent Agrionid nymphs under the microscope a stream of water taken into and expelled from the rectum. Tillyard (1906, p. 415) recorded the larva of *Lestes leda* as suddenly projecting "itself forward by expelling the water from its body anally," which certainly suggests two functions, locomotion and respiration, associated in the rectum as they are in Anisopterous larvae. Babak and Foustka (1907, p. 538), studying the respiratory movements of the abdomen of Odonate larvae by means of the graphic method, obtained from "a small species of the Agrionid group" results entirely similar to those from experiments on two larger Libellulid species. Their figures show the differences in the height and frequency of the respiration curves, due to the absence or presence in varying quantities of oxygen in the water in which the larva was placed. Balfour-Browne (1909, p. 279) working on larvae of *Agrion pulchellum, Ischnura elegans, Pyrrhosoma nymphula* and *Erythromma najas,* after citing Dewitz's observation quoted above, says of the stream of water passing in and out through the anus:

> from my observation it is a very weak one, nor is there any special apparatus surrounding the anus in this group to prevent ingress of foreign particles such as is found in the Anisopterids. If rectal respiration exists at all, it seems to me that it must be very slight and of but little importance, as I could not observe any increased number of contractions of the rectum in specimens of *Agrion* which had been deprived of their lamellae. In the absence of the lamellae, I think the whole of the respiration must be carried on through the skin.

It is remarkable that, as in the case of *Calopteryx,* no anatomical-histological studies have been made on the rectum of Agrionine larvae of Europe or of North America. All that exists hitherto appears to be the brief account of the rectum of the Costa Rican *Mecistogaster modestus* (Calvert, 1911*b,* pp. 452-3, pl. xvii, ff. 7, 10). The first part of the present paper gives a fuller description, of the rectal tracheal distribution at least, than any that has yet appeared.

Observation and experiment with carmine particles on living larvae of *Argia moesta putrida* (dimensions as before: 7.5, 3 and 4 mm., and smaller), from the vicinity of Philadelphia, showed that a larva watched for two hours may give no sign of *rhythmic* rectal contractions, but that these may suddenly begin and then continue for varying periods. They consisted of sets of three rapidly successive contractions, every third contraction being of longer duration than the other two; 32 such sets per minute were noted. At other times sets of five contractions were noted. In some larvae, but not in all, the beginning of a set of rectal contractions was often synchronous with a slight shortening of the abdomen; the lengthening of the abdomen began before the contractions had ended. A rhythmic shortening and lengthening of the abdomen may perhaps furnish an indication of the existence of rectal contractions in opaque larvae. Successive removal of the three caudal gills in one larva was not followed by any immediate beginning of rectal contractions, although such were seen four hours later; the larva was not under continuous observation during all of that time, however. The observations of the *Argia* larvae were made chiefly under a Zeiss compound microscope, objective A, ocular 2.

LATERAL EXTERNAL ABDOMINAL TRACHEAL GILLS

are now known for the following Calopteryginae:*

Euphaea splendens. Hagen, C. R. Soc. Ent. Belg, xxiii, p. lxvi, 1880; Zool. Anz. iii, pp. 160, 304, 1880.† Packard, Text-Book Ent. pp. 468-9, fig. 446, 1898.

Euphaea variegata. Ris, Tijdschr. Ent. lv, p. 168, pl. 6, figs. 9, 15-20, pl. 7, figs. 10-12, pl. 8, figs. 13, 14, 1912.

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*The references are to descriptions of the larvae only.

†Hagen states (*l. c.* p. 160): "Die ersten derartigen Larven erhielt ich von Nietner in Ceylon und habe über sie mündlich in der Naturf.-Versamml. in Stettin 1861 berichtet." The Versammlung der deutscher Naturforscher und Aerzte in Stettin was in 1863, not 1861. The Bericht of that [38th] Versammlung for September 23, 1863, p. 137, reads "Herr Dr. Hagen über Respirations-Organe von Euphaea splendens, woran sich eine Discussion schloss, an der sich besonders Director Loew und Prof. Grube betheiligten," but nothing more. The Versammlung of 1861 was at Speyer and the Bericht thereof contains nothing by Hagen and nothing on *Euphaea.*

Anisopleura comes? Needham, Ent. News, xxii, p. 149, pl. v, figs. 1-3, 1911.

Euphaea dispar? Hagen, C. R. Soc. Ent. Belg., xxiii, p. lxvi, 1880.

Bayadera indica. Needham, Ent News, xxii, p. 150, pl. v, figs. 4-7.

Anisopleura comes? Hagen, C. R. Soc. Ent. Belg., xxii, p. lxvi, 1880.

Cora chirripa. Calvert, Ent. News, xxii, p. 49, pls., ii, iii, 1911.

It is not stated whether these lateral gills are kept in movement during life or not.

STIGMATA (SPIRACLES).

Dewitz (1890, pp. 504, 526), on immersing Agrionid nymphs in diluted alcohol, or on raising or lowering the temperature of the water in which they were contained, saw air escape from a thoracic stigma of one side of the body. As the older nymphs crawled up to the surface of water, which had been boiled, and exposed their dorsal thoracic surface, he inferred that they inspired atmospheric air.

By subjecting the early stages of Odonata to the action of oleo-ether and to partial vacua, Portier (1911, pp. 216-7) concluded that in *Calopteryx,* as in *Aeschna* and *Libellula,* the tracheal system is not closed to air, although not permeable to water, fats or their solvents. In the younger *larvae,* one or the other of a pair of ventral spiracles at the junction of thorax and abdomen allows passage to air, while in the older *nymphs* these ventral stigmata are lacking, but one or other of a pair of anterior dorsal thoracic [mesothoracic] spiracles transmits air from within or from without.

Bervoets (1913, pp. 25-26) also employed the vacuum method. In larvae of *Agrion* 3 mm.* long he was unable to perceive any discharge of gas until he cut the caudal gills; in larvae 5 mm.* in length and longer, bubbles of gas issued from the dorsal stigmata between the pro- and meso-thorax, always more abundantly from one side than from the other.†

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*These dimensions were exclusive of the caudal appendages.

†Bervoets has used (pp. 27, 29) the term *Isoptera* for the Agrionidae, evidently in contrast with Anisoptera. Isoptera is, of course, preoccupied as an ordinal name for the Termites.

These experimental researches on the permeability of the spiracles to air may afford an explanation of the results obtained by East (1900, p. 212), who kept nymphs of *Erythromma najas* and of *Agrion puella* alive, out of water, for one month and for thirty-three days, respectively. They had no food during this period and yet the nymph of *E. najas* moulted in the midst of it.

CONCLUSION.

From the various data which have been brought together here, it seems reasonable to suppose, at least until much more exact experiments show the limitations of each mode of respiration for different stages and for different species of Zygopterous larvae, that the general body-surface, the caudal processes, the rectal epithelium, certain spiracles and, in a few species, lateral external abdominal tracheal gills, *all* contribute to satisfying the needs of the organism for oxygen. A classification which regards the larvae or nymphs of any one group as exclusively or predominantly Caudobranchiate is not justified by our present knowledge. The fact that the larvae of *Thaumatoneura* spend much of their lives moistened by, but not submerged in, water, tempts one to refer the relatively small surface of their caudal appendages ("gills") to this mode of life. The similarity of their rectal tracheation to that of the larva of *Hetaerina americana* and the existence of rectal respiration in the latter lead one to expect that a similar condition will be demonstrated by observations on living *Thaumatoneura* larvae.

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Studies on Costa Rican Odonata.

VIII. A New Genus Allied to Cora.

By Philip P. Calvert, University of Pennsylvania,
Philadelphia, Pa.

In our recent book on Costa Rica* we have referred (page 255) to the capture, at Peralta, August 8, 1909, of "a medium-sized dragonfly, of an apparently new genus allied to *Cora*." Only a single male was obtained in spite of search in the same locality on following days in August and in March, 1910. The insect is evidently distinct from *Cora*, although the latter is its closest known ally. Mr. E. B. Williamson has very recently obtained a long series of the same genus, and possibly the same species, in Colombia, some specimens of which he has kindly sent me for examination, so that the recognition of this form as generically distinct seems thoroughly justified. The following account is based on the Costa Rican specimen, leaving to Mr. Williamson the future description of his Colombian material.

MIOCORA† new genus.

Possessing these characters of the legion Thore of Selys, viz.: Upper and lower sectors of the arculus (M1-3 and M4) separating from the upper (anterior) end of the arculus; proximal side of the quadrilateral much longer than the distal, its anterior (upper) side concave, costal and subcostal series of antenodals subequal in number but not coinciding in position in most cases; quadrilateral and median cell (M) cross-veined, the former shorter than the latter; and the following characters of the genus *Cora* Selys, viz.: M3 unbranched, no supplementary sectors between M4 and Cu1, and only one antenodal cross-vein (here the 8th or 9th, front wings, 9th or 10th, hind wings, of the costal series) thicker than the others.‡

---

*A Year of Costa Rican Natural History by Amelia Smith Calvert and Philip Powell Calvert, New York. The Macmillan Company, 1917.

†Greek μείων, less, and *Cora*, in allusion to the reduced venation in comparison with that genus.

‡In the Colombian specimens the thickened antenodal varies from the 9th to the 14th, front wings, 8th to 13th, hind wings.

Its distinctive differences from *Cora*, and indeed from the whole legion Thore of de Selys (1869), are the unbranched condition of Cu2, the presence of only a single row of cells between Cu1 and Cu2, and of only a single row between Cu2 and the hind margin of the wing.

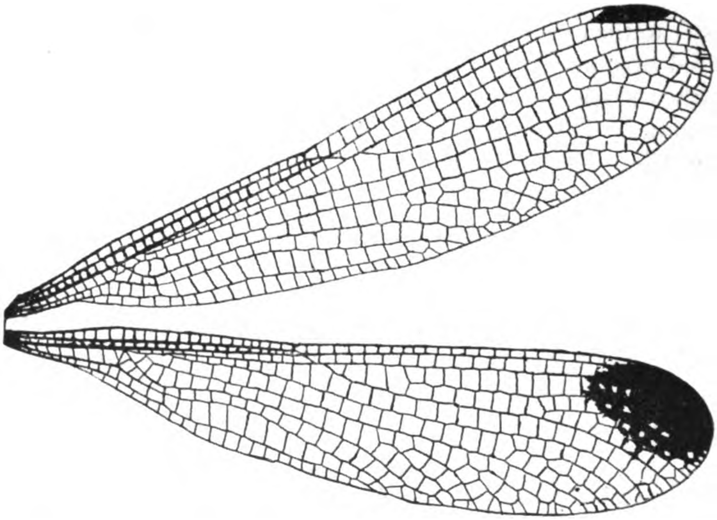

Fig. 1.—Venation of *Miocora peraltica* ♂, Peralta, Costa Rica, August 8, 1909. From photograph by Mr. H. A. Walters. See text for an explanation concerning the front wing.

The venation is displayed in the accompanying figure where, owing to the longitudinal folding of the front wing, M1-3 and M1-2 for a short distance distal to the separation of M3 is not shown as distinct from R, as it actually is in both front and hind wings, and as shown in our figure of the hind wing.

Genotype: *Miocora peraltica* n. sp.

Miocora peraltica n. sp. (Text figs. 1-4).

♂. Colors (as noted from the freshly caught specimens, some additional details not mentioned in the field notes but visible in the dried specimen being added in brackets [ ]): Eyes dark brown, below somewhat bluish. [Remainder of the head black, a short orange streak between each antenna and the median ocellus but nearer to the antenna], labrum, genae and external surfaces of the mandibles light blue, [cardines, stipites, submentum, mentum and basal two-thirds of median labial lobe pale luteous, probably also light blue in life.].

[Prothorax black, a pale blue? spot on each side of the middle lobe.]

Thoracic dorsum blackish; humeral suture, most of metepisternum and much of metepimeron pale bluish-green, but metepisternum and metepimeron each with an [oblique] blackish stripe, less than one-half as wide as the metepisternum but more than one-half as wide as the metepimeron. [Pectus pale bluish-green.]

Abdomen black, a spot on each side of segment 1, a longitudinal stripe on each side of 2 and a small basal spot on each side of 3, pale green.

Ventral surface of thorax pruinose and traces of pruinosity on ventral surfaces of abdominal segments.

[Legs: femora superiorly, tibiae inferiorly and tarsi blackish brown, femora inferiorly and tibiae superiorly pale, perhaps bluish in life.]

Superior appendages twice as long as abdominal segment 10, hardly as long as segment 9, black, in dorsal view forcipate, each becoming more slender to the apex which is acute and bent mesad at the extreme tip. Each appendage has a straight inferior process, projecting mesad, ventrad and caudad, best seen in oblique dorso-lateral view, and whose proximal and distal edges separate from the inferior margin of the appendage at 5/14 and 8/14 of the latter's length respectively; distal edge of this process 3/14 of the length of the appendage; process becoming more slender toward its roundly acute apex. In profile view each appendage is directed caudad and ventrad as far as the last tenth of its length where its acute apex is curved dorsad; the appendage gradually decreases in thickness from base to apex, shows on its inferior margin a slight post basal convexity and the process described above, foreshortened at about mid-length. Inferior appendages not developed.

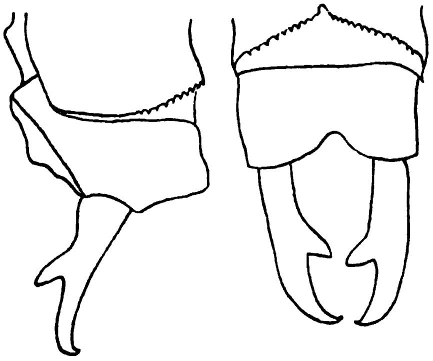

Fig. 2.—Left profile, Fig. 3.—Dorsal
Views of apex of abdomen of *Miocora peraltica* n. sp., type ♂.

Front wings: 26 to 28 antenodals, 24 to 25 postnodals, 10 cross-veins in the median space, 3 cross-veins in the quadrilateral, pterostigma surmounting five cells and parts of one or two others, its proximal edge twice as long as its distal edge.

Hind wings: 25 to 22 antenodals, 24 to 26 postnodals, 8 to 9 cross-veins in the median space, 4 cross-veins in the quadrilateral, pterostigma surmounting four cells and parts of two others, its proximal edge 1.6 as long as its distal edge.*

All wings faintly smoky, costal and subcostal areas faintly yellowish from the base distad to beyond the nodus. Hind wings with an apical brown spot extending from about two cells proximal to the proximal end of the stigma to the wing-apex and from the costal margin to M_2, some cells between M_2 and R*s*, also being faintly brown, many of the cells within the area of the spot paler in their centers. Pterostigmata dark reddish brown.

Total length 42, abdomen 34, superior appendage 1.16, hind wing 24.5,

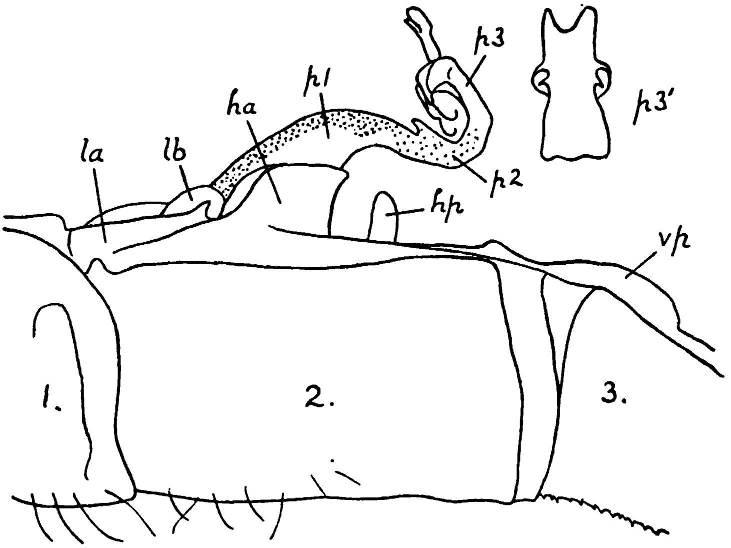

Fig. 4.—Genitalia of the basal abdominal segments, *Miocora peraltica*, n. sp., type ♂. The abdomen is viewed from the right side, ventral surface uppermost. 1, 2, 3, abdominal segments 1, 2 and 3; *ha*, *hp*, anterior and posterior hamules; *la*, anterior lamina; *lb*, lamina batilliformis of Rathke (1832) and Schmidt (1915), sheath of the penis of Rambur (1842) and of American authors; *p1*, *p2*, *p3*, first, second and third segments of the penis of Kennedy (1916); *p3'*, third segment in dorsal view; *vp*, vesicle of the penis ("Samenkapsel").

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*As might be expected, the Colombian specimens show some variations in the numbers of these veins and surmounted cells in both front and hind wings.

costal edge of stigma of front wing 2, of hind wing 1.8, maximum width of front wing (mid-way between nodus and stigma) 5.5, same of hind wing, 5.5, width of head 4.8 mm.

Type a male from Peralta, Costa Rica, August 8, 1909, by P. P. Calvert, in the writer's collection at the Academy of Natural Sciences of Philadelphia.

In August, 1909, a short distance back of Peralta Station of the Costa Rica Railway, altitude 322 meters, 1055 feet, there extended a Y-shaped track to enable locomotives to reverse their heading. At the end of the stem of the Y was a narrow, slow-moving stream called simply "laguna." Just beyond the laguna was a low woods consisting of small trees, arums, ferns, heliconias and numerous vines or creepers. Here the type of *Miocora peraltica* was taken about noon.

The generic name *Thore* Selys, 1853, from which his legion Thore takes its appellation, was preoccupied by the name *Thore* applied to a subgenus of Attid spiders by C. L. Koch (Übersicht des Arachnidensystems, 5tes Heft, p. 66, Nürnberg, 1850). I therefore propose the name **Polythore** for the Odonate genus in allusion to the denser venation of its members in comparison with other genera of the Selysian legion. The type of *Thore* was fixed by Kirby (Cat. Odon. 1890, p. 116) as *T. gigantea* Selys, so this species becomes the genotype of *Polythore*. The oldest generic name thus left in the legion is *Chalcopteryx* Selys, 1853.

UNIVERSITY OF PENNSYLVANIA

THE SEGREGATION AND RECOMBINATION OF HOMOLOGOUS CHROMOSOMES AS FOUND IN TWO GENERA OF ACRIDIDAE (ORTHOPTERA)

BY

E. ELEANOR CAROTHERS

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF PENNSYLVANIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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E. ELEANOR CAROTHERS

From the Zoological Laboratory of the University of Pennsylvania[1]

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[1] Contribution from the Zoological Laboratory of the University of Pennsylvania.

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I. INTRODUCTION

1. *Statement of the problem*

The present trend towards a closer correlation of the subjects of cytology and genetics is one that promises much mutual benefit, as is well demonstrated by the work of T. H. Morgan and his co-laborers at Columbia University. While the present work deals entirely with cytological data, the material promises much for a genetical study which, it is hoped, may be undertaken later.

Early in the course of his studies on Acrididian spermatogenesis Dr. McClung outlined a general plan of. study which, it is hoped, will lead to a correlation between chromosomes and body characters. This plan involves (1) a study of the chromosomes of selected species of closely related genera; (2) a study of numerous species within a genus; and (3) a study of the details of organization of the chromosomes of certain species.

In pursuance of this plan McClung has worked on the genus Hesperotettix; Nowlin, taking up the genus Melanoplus belonging to the same group, has published ('08, '14) her results on five species of this genus; Robertson ('16) has carried on work on the Tettiginae; Pinney ('08) and Wenrich ('16) have studied the genus Phrynotettix; and the writer has begun work on the genus Trimerotropis—subfamily Oedipodinae.

It is my purpose to confine this paper as closely as possible to its own special topic. Two hypotheses, put forward by Van Beneden ('83) and elaborated by Boveri ('88, '02) are, however, essential to an interpretation of the facts presented. The first, that of the individuality of the chromosomes, also supported by Rabl ('85), has been actually demonstrated for the individual.

at least, in one of the short-horned grasshoppers, Phrynotettix magnus, by Wenrich ('16) who, through years of close study, has been able to follow one well marked individual chromosome pair from the spermatogonia through the most diffuse stages to the spermatids. The other one-time hypothesis of Van Beneden ('83) that one-half of the chromosomes are of paternal and one-half of maternal origin was clearly shown to be a fact by the late Carl Mulsow ('12) in his study of the gametogenesis and fertilization of a parasitic trematode, Ancyracanthus, in which the chromosomes may be counted, even in the living spermatozoon as it enters the egg and fuses with the female pronucleus.

Sutton ('02) was the first to furnish actual support for the latter hypothesis and to suggest a thorough-going correlation of the chromosomes and somatic characters; he recognized in Brachystola magna a double series of chromosomes in the spermatogonia which he considered to be of biparental origin. He indicated the probable relationship between Mendelian phenomena and the possible distribution of the chromosomes in the gametes and their recombination in the zygote. But, so long as the homologous chromosomes of this double series were indistinguishable, it was impossible to follow, cytologically, the chromosomes derived from a given parent and to determine their manner of segregation in relation to any other chromosomes.

As is well known, in the Orthoptera and many other animals as well, the first maturation division determines which of the derivative cells will, in fertilization, produce a male and which a female, since the accessory chromosome—the sex determinant—passes undivided into one of the daughter cells at this time. While probably it is purely a matter of chance which of the daughter cells the accessory enters—that is, it parallels any Mendelian character in the matter of segregation—nevertheless, it marks unalterably, after it has passed to one pole, the male from the female producing spermatozoon. If one of the homologues of any pair is recognizably different from its mate and these homologues should segregate from each other at the first maturation division their manner of segregation in relation to sex would be apparent. The only instances of this sort to be reported, so far, are those in which the homologues differ markedly in size. The first was the work of the writer which appeared in 1913, giving data showing the alternative distribution of the unequal dyads of one tetrad in relation to sex in certain Oedipodinae. Three hundred first spermatocytes were counted; in 51.3 per cent the larger dyad was going into the same second spermatocyte as the accessory, and in 48.7 per cent the smaller dyad was accompanying the accessory. Shortly afterward a paper by Voïnov ('14) appeared, giving like results and an essentially similar conclusion from a study of Gryllotalpa vulgaris.

Wenrich, in 1914, reported similar results from a count of four hundred and seventy-two first spermatocytes of Phrynotettix magnus where a tetrad with unequal dyads is also present. And lastly, Robertson ('15), in an addendum to a paper in which he reports an unequal pair of chromosomes in Tettigidea parvipennis and in Acridium granulatum, states that these unequal pairs agree with the one in my material in regard to the distribution of their dyads in relation to the accessory. These I believe are the only instances so far reported where the chromosomes derived from different parents could be followed in the germ cells and their distribution determined.

It is to this problem of tracing the segregation of certain well marked homologues in given individuals of the species studied and then of determining the combinations actually present in a considerable number of individuals of these species that I wish to devote this paper. My study has been confined to the metaphases, but a knowledge of the behavior of the dissimilar homologues during synapsis is essential to my conclusions. As this problem was aside from my general plan, and directly in line with Dr. Wenrich's work, he kindly undertook its solution. In a paper now in press he shows that the process of synapsis and tetrad formation is the same for the heteromorphic tetrads as for the homomorphic ones, whether the latter have terminal or nonterminal fiber attachment.

2. *Nomenclature*

The unusual conditions of the chromosomes in this group have made advisable the introduction of four new terms.

1. Homomorphic—used to designate those tetrads made up of morphologically similar homologues.
2. Heteromorphic—used to designate those tetrads made up of morphologically different homologues.
3. Telomitic—a term used to indicate terminal fiber attachment.
4. Atelomitic—a term used to indicate nonterminal fiber attachment.

The two latter are extensions of the ideas involved in the terms 'Hippiscus type' and 'Stenobothrus type' as used by McClung ('14). This has seemed desirable since the work of Robertson ('16) and some recent work of McClung indicates that the chromosomes of Stenobothrus may be fundamentally different from those of the typical Acrididian complex. I shall also apply the terms telomitic and atelomitic to spermatogonial and somatic chromosomes as well as to those of the first spermatocyte. The term Hippiscus type will be used interchangeably with telomitic for those first spermatocyte chromosomes which are comparable in structure to the Hippiscus rings. However, in this material the tetrads of the Hippiscus type are usually transformed into rods in the metaphase as described by McClung ('14). In the same way Stenobothrus type and atelomitic may be used interchangeably.

3. *Special fitness of material*

The genus Trimerotropis is confined to the American continent and contains over thirty species, the extreme members of which merge with those of four other genera. The present paper deals with the fallax group of the genus and its relation to the genus Circotettix, with especial reference to the heteromorphic tetrads found in each. In regard to the systematic relationships, it is believed that a definite cytological criterion—

difference in number of chromosomes—has been found for separating the two genera.

On this evidence the species suffusus belongs to the genus Trimerotropis instead of to Circotettix, while the heteromorphic tetrads found in both genera may point to a common origin. Furthermore, the evidence, as will be shown, indicates that the fallax group constitutes a single species.

The group which forms the basis of this work is known to taxonomists as one of the most difficult to classify. Certain species of the group have been removed to the genus Circotettix by some taxonomists, while others have split up the remainder into four species. If one assume that the chromosomes are direct descendants from ancestors morphologically like themselves, are the bearers of the determiners of the hereditary characters, and that the homologues actually express, in their architecture, differences in the determiners, the results of a study of the maturation divisions in the male leave little surprise that taxonomists are bewildered.

Dr. McClung has shown ('14) that the point of spindle fiber attachment is normally constant, but at some period in the history of this species there must have been a reorganization to the extent of a shifting of the attachment in certain chromosomes. The most striking result has been to produce J-shaped tetrads in the first spermatocyte. Such shapes are due to one homologue being rod-shaped; that is, it has terminal fiber attachment, while its mate has nonterminal fiber attachment. Of the twelve first spermatocyte chromosomes, ten tetrads and the accessory are affected by this reorganization. If the female is similarly involved and if there is free fertilization, bringing about chance recombination, one might expect to find in a given individual all ten of the tetrads represented by two rod-shaped homologues, giving rod-shaped tetrads, or one might find all of the homologues with nonterminal fiber attachment, resulting in tetrads of the Stenobothrus type (McClung '14). Between these two extremes there might be present every possible combination in J's, rod's and Stenobothrus-like rings.

These expectations have been realized in part; and, furthermore, in two of these ten tetrads there is a third type with both homologues having nonterminal fiber attachment, but with a secondary shifting so that one of the free arms is longer than the other. One finds, besides such heteromorphic tetrads (plate 14, fig. 22*c*), homomorphic tetrads of the two expected types, one with both arms long (plate 14, fig. 23*a*) and the other with both arms short (plate 14, fig. 10*a*).

Another peculiarity, that of a single constriction occurring near the proximal ends (that is, the ends directed towards the poles), may mark certain tetrads. An example may be seen on plate 14, figure 21*a*. One dyad of such a tetrad (Chromosome number 3, plate 4) may be of three types; V-shaped (plate 5, fig. 31), plain rod (plate 5, fig. 32) or constricted rod (plate 5, figs. 31 and 32); these occur in various combinations, furnishing a visible mechanism for possible triple allelomorphs.

4. Methods and acknowledgments

The material for the somatic mitoses of the female was fixed in a modification of Bouin's solution which has been developed in this laboratory and which promises to be very useful. The following is the formula:

Picric acid, saturate aqueous solution	75 cc.
Formalin (full strength)	15 cc.
Glacial acetic acid	10 cc.
Urea, crystals	½ gm.

After immersion in this fluid, material may be removed any time between twenty-four hours and three months. It should then be washed in water some fifteen minutes and run up gradually to 75 per cent alcohol where it may be kept indefinitely. The fact that specimens may be left for a long time in this fixative makes it especially desirable for field work and for those not equipped to make use of more elaborate methods. Acrididian metaphase chromosomes are as well preserved as in Flemming.

The testes were fixed in strong Flemming solution. Sections were cut from 7 to 12 microns in thickness; 10 proved best suited to this work. The stains used were Flemming's tricolor and Heidenhain's iron-hematoxylin.

I wish to thank Dr. McClung for many helpful suggestions and much kindly interest as well as for material from his own collection. It is also through his efforts that I am indebted to the University of Pennsylvania for a collecting trip during the summer of 1915 to obtain additional material. I am, besides, indebted to the Marine Biological Station at Woods Hole for laboratory facilities during the summer of 1914. I also wish to express my thanks to Mr. J. A. G. Rehn and Mr. Morgan Hebard of the Academy of Natural Science for the identification of the specimens and helpful suggestions concerning the taxonomy of the group, and finally to Dr. D. H. Wenrich for much kindly cooperation.

II. OBSERVATIONS

Since the evidence for segregation and recombination must be derived largely from the maturation divisions, I shall begin with the first spermatocytes, then go back to the spermatogonia and to the somatic complexes of the female for corroborative evidence, and finally, take up the second spermatocytes, which are morphologically related directly to the spermatogonial and somatic complexes. The individuals are numbered consecutively. Where more than one complex is shown from an individual the succeeding ones are indicated by letters; e.g., number 1 always refers to a given animal; the letters after the 1 (*a to x*) designate drawings from this specimen.

1. First spermatocytes

a. Segregation of homologues of J-shaped tetrads in individual no. 1, Trimerotropis (?) suffusa (?). This is one of two individuals belonging to what I designate as form 'B.' They were collected on Orcas Island in Puget Sound by a party from Kansas University in the summer of 1909. Both individuals are alike.

Of the twelve first spermatocyte chromosomes (plate 1), four (nos. 9 to 12) are atelomitic—Stenobothrus type (McClung, '14)—four (nos. 2, 3, 5, and 6) are telomitic—Hippiscus type, the accessory (no. 4) is V-shaped, atelomitic, while the remaining three tetrads (nos. 1, 7 and 8) have one dyad of the Stenobothrus type and the other of the Hippiscus type.

Several important questions at once present themselves. 1) Since these heteromorphic but homologous dyads segregate in the first maturation division, what is their distribution in relation to the accessory (that is, to sex)? 2) As it is obvious that at least sixteen sorts of spermatozoa are formed in these two individuals, would the well known constancy of the complex—as shown by numerous workers on Orthoptera—hold for this species or would a large number of individuals give all possible combinations? Or would certain combinations result and others fail? 3) Would the somatic complex of the female be constant? (Using this as an index of the oogonial complex). 4) If the complex is constant for the species, what is the mechanism by which it is regulated? Is there selective fertilization of a most complex sort, or is there free fertilization with regulation occurring at the time of the maturation of the egg? (Since copulation occurs some twenty-four hours before the polar bodies are formed the latter seemed quite possible.)

The first of these questions was a matter to be determined by a study of a given individual and was at once worked out from the material in hand. The others required a considerable number of individuals and it was largely for the purpose of obtaining these that a collecting trip through the southern and western states was undertaken during the summer of 1915.

For a determination of the method of segregation of the heteromorphic homologues in relation to the accessory, one hundred camera lucida drawings of entire complexes were made at random. As stated above, the two individuals in the collection were alike; fifty-seven of the drawings were from one individual and forty-three from the other. Plate 1 is based on only one individual, however, but is representative of both.

These drawings are from sections. The chromosomes from one cell are always in two and sometimes three sections. The sections were all present, in order and in straight rows, so that the problem of identifying in successive sections these large clear cells with sharply formed spindles is much simpler than it may appear to those accustomed to less favorable material. The chromosomes were first outlined under the camera lucida in their actual relation to each other in the sections, then a careful study was made and details filled in when necessary. Later, the chromosomes represented in plate 1 were arranged roughly according to size and placed on the plate so that the transverse rows represent the chromosomes found in one cell while the vertical rows represent the corresponding chromosomes in different cells. The eight complexes shown on this plate are typical of the conditions found in the hundred cells. They are so placed that the accessory is always passing to the upper pole.

Taking up first the small one at the right (no. 1), which is one of the heteromorphic pairs, we find in five of the cells (*b*, *c*, *d*, *f*, *h*) the atelomitic dyad going to the cell which lacks the accessory, while in three (*e*, *g*, *i*) it is going into the same cell as the accessory. The other two heteromorphic chromosomes, numbers 7 and 8, respectively, are so nearly identical in size and behavior that no attempt was made to distinguish between them. Instead, the segregation of their homologues in relation to each other and in relation to the accessory was noted and gave all of the information desired. If these two chromosomes are compared with each other in the first four cells (*b*, *c*, *d*, *e*), it will be observed that the dyads with nonterminal fiber attachment are going to opposite poles; in the remaining four cells (*f*, *g*, *h*, *i*) they are going to the same pole. But in cells *f* and *g* they approach the pole which lacks the accessory, while in the last two they will enter the same cell as the accessory. Now, if we compare chromosome number 1 with numbers 7 and 8, it will be seen that its dyads also segregate independently of either of the others. For instance, in the last four cells (*f*, *g*, *h*, *i*) its atelomitic dyad passes, either to the same second spermatocyte as the similar dyads of the larger chromosomes (*f* and *i*), or to the cell which receives the telomitic dyads (*g* and *h*). It is evident then that here are four chromosomes (nos. 1, 4, 7, 8) for which this is the segregation division and that they are distributed more or less without regard to each other or to the second spermatocytes.

If there is free segregation, the number of equally possible combinations in the gametes of a single individual is represented by the formula 2^n in which n represents the number of chromosomes in the reduced series; that is, the number of pairs of homologues. In this instance $n = 4$ since we are considering only the accessory and the three heteromorphic tetrads. Then 2^4, or 16, is the possible number of combinations of these chromosomes in the gametes of this individual. While the number of morphologically different gametes formed as a result of the segregation of any *given* three is 8 (2^3), of any given two is 4 (2^2) of a given one 2 (2^1). The occurrence of *any* combination is shown by the coefficients of the expanded binomial raised to the nth power in which case n again represents the number of homologous pairs. In this instance the series of coefficients is 1–4–6–4–1.

From the two formulae given above we should expect to find

Any *given* 4 V's in $\frac{1}{16}$ of the gametes	*Any* 4 V's in $\frac{1}{16}$ of the gametes
Any *given* 3 V's in $\frac{1}{8}$ of the gametes	*Any* 3 V's in $\frac{4}{16}$ of the gametes
Any *given* 2 V's in $\frac{1}{4}$ of the gametes	*Any* 2 V's in $\frac{6}{16}$ of the gametes
Any *given* 1 V in $\frac{1}{2}$ of the gametes	*Any* 1 V in $\frac{4}{16}$ of the gametes
and 0 V in $\frac{1}{16}$ of the gametes	and 0 V in $\frac{1}{16}$ of the gametes

The difference—it will be noted—between these two series is in regard to *any* one, two or three as opposed to a *given* one, two or three; that is, in the latter case, we must distinguish between the V's with which we are dealing. A given V (as the accessory) would be found in one-half of the gametes, but, on the other hand, one-quarter of the gametes would contain only one V.

Perhaps both may be better shown graphically. Let A, B, C, D, represent the V-shaped homologues and the accessory and a, b, c, d, the rod-shaped homologues of the tetrads and the absence of any homologue in the case of the accessory. There are then the foregoing sixteen combinations any one of which is equally probable; *all* four V's together, e.g., A B C D, one-sixteenth of the time; *any* three, as in the second division, four-sixteenths of the time; any *given* three, e.g., A, B, C, two-sixteenths of the time; *any* two, as in the third division, six-sixteenths of the time; any *given* two, four-sixteenths of the time; while any *given* one occurs eight-sixteenths of the time and *only* one four-sixteenths of the time.

A B C D	a B C D	a b C D	a b c D	a b c d
	A b C D	A b c D	a b C d	
	A B c D	A B c d	a B c d	
	A B C d	a B c D	A b c d	
		a B C d		
		A b C d		

To be specific if A = accessory, B the V-shaped dyad of number 1 and C and D the indistinguishable ones of numbers 7 and 8, then, out of sixteen second spermatocytes, we should have the accessory in eight-sixteenths, the accessory and V of tetrad number 1 in four-sixteenths, any two V-shaped dyads six-sixteenths, any three in four-sixteenths. As the one hundred spermatocytes counted represent two hundred derivative cells, we have:

	Expected	*Actual count*
A given V (Accessory or no. 1)	$\frac{1}{2}$ x 200 = 100	100
Only one V..	$\frac{1}{4}$ x 200 = 50	48
Two given V's (Accessory and no. 1 or those of 7 and 8)	$\frac{1}{4}$ x 200 = 50	46 and 47
Any two V's.......................................	$\frac{3}{8}$ x 200 = 75	84
Three given V's (nos. 1, 7, 8 or accessory, 7, 8)	$\frac{1}{8}$ x 200 = 25	22 and 21
Any three V's.....................................	$\frac{1}{4}$ x 200 = 50	48
All four V-shaped.................................	$\frac{1}{16}$ x 200 = $12\frac{1}{2}$	8

Considering that these figures are based on only one hundred first spermatocytes, the results are probably as near the expectations as could be anticipated for any objects on the basis of chance distribution for a like number of trials.

b. Recombination of the homologues of the J-shaped tetrads in eighty-two individuals of Trimerotropis (?) fallax (?). The use of the word recombination may require some explanation. The two dissimilar homologues composing a heteromorphic tetrad are segregated into different male gametes. For example the atelomitic dyad of a J-shaped tetrad goes to one pole and the telomitic dyad to the opposite pole. Gametes carrying each kind of homologue would therefore be formed. If we may assume that the form of the homologues remains constant and that a female may carry J-shaped (heteromorphic) tetrads and produce different types of gametes, in respect to such chromosomes, like those of the male; then, if free fertilization occurs, there should be found in the offspring all possible recombinations of the homologues; e.g., tetrads with two atelomitic dyads and tetrads with two telomitic dyads as well as J-shaped tetrads. The only way to demonstrate that such recombination occurs is through breeding experiments. But since I have collected at random a considerable number of individuals (eighty-two) and have analyzed their first spermatocyte complexes, and have found the types of chromosomes which would be expected from the above assumptions, I have ventured to use the term recombination to express the relationship of those different types to each other.

It is obvious from the above section, that the two individuals there considered formed sixteen sorts of spermatozoa, so far as the selected elements are concerned. A study of a comparatively large number of animals was undertaken for the purpose of finding, in the first place, whether or not the complex is constant for the species and, secondly, if not, to determine the range of variation. The results from sixty-two individuals are given in plates 2 to 9. The chromosomes on these plates are arranged in the same manner as on plate 1, except that only one complex from an individual is shown; therefore each of the horizontal rows represents one animal. There are two exceptions to this statement: number 32*a′* is a spermatogonial metaphase from the same individual as number 32, and number 63*a′* is a spermatogonial metaphase from animal number 63.

It will be seen from plate 1 that animal number 1 has, as already stated, in addition to the three J-shaped tetrads, four atelomitic and four telomitic chromosomes. Such a complex was found to be constant for two individuals. This statement is based on one hundred camera lucida drawings. Only one complex is shown from the succeeding animals, but a comparative study of a number of complexes was made in each case so that it can be safely stated that the complex is practically constant in any one individual.

Taking up animal number 2 (plate 2), we find five atelomitic tetrads, numbers 7, 9, 10, 11 and 12; one heteromorphic tetrad, number 1; and the remaining five telomitic. Passing on to animal number 3 we find six atelomitic, three heteromorphic (J-shaped) and only two telomitic tetrads. Chromosome number 1 which in the preceding animals has been heteromorphic has both homologues of the atelomitic type. If we study individual number 6 we find that it has no J-shaped tetrads; it has eight atelomitic and only three telomitic tetrads. Comparing individuals 6 and 7 it is seen that chromosomes number 9 to 6 inclusive are atelomitic in the former and J-shaped in the latter.

It is also interesting to compare more in detail individuals number 2 and number 6. Chromosome number 1 in individual number 6 is the type which would be expected to result if the spermatozoon carrying the V-shaped dyad of this chromosome in animal number 2, for instance, united with an egg bearing a similarly shaped dyad. The shape of chromosomes number 6 and number 8 of animal number 6 may be accounted for on a similar assumption. Conversely we would expect to find, then, in some individual the two telomitic homologues of these two tetrads, and this is exactly what we have in animal number 2.

Clearly, then, the complex is not constant in this species, according to our past conceptions of constancy as indicated by form of chromosome, since a given chromosome in one member of this species may be of the Stenobothrus type, in another of the Hippiscus type, while in a third, one homologue of this same chromosome is of the Stenobothrus type and the other of the Hippiscus type. This is most easily demonstrated by following chromosome number 1 through several plates. Taking plates 2 and 3 we find this element of the mixed type in eight of the sixteen individuals, while it is of the Stenobothrus type in four and of the Hippiscus type in four. For the entire sixty-two individuals under consideration this tetrad is of the Stenobothrus type thirteen times, Hippiscus type eighteen times, one homologue of each type thirty-one times. This comes very close to the 1-2-1 ratio that we might expect on Mendelian principles if the two types occur with equal frequency in nature and chance fertilization occurs.

The next point was to determine, so far as my material permitted, the range of variation. I wish to emphasize the statement previously made that the arrangement in the plates according to size is only a rough estimate. Numbers 1, 2 and 4, I feel sure, are correctly identified in all cases, but aside from these there are scarcely any two consecutive ones which might not exchange places, yet the extremes are clearly distinguished. As the chromosomes are arranged, numbers 10, 11 and 12 are rings of the Stenobothrus type in all cases. I am inclined to think that this is correct. However, chromosome number 9 of the fifth animal might well change places with number 11. I have not been able to estimate satisfactorily the quantity of chromatin in the rings. On the other hand, it is readily seen on the slides that a given chromosome may be stretched out considerably with a comparatively slight thinning to compensate. The difference between an early and a late metaphase is also very striking. In the former the chromosomes are short and thick while in the latter they are evidently coming under some force which tends to stretch them out along the spindle axis. There is some evidence that this force is exerted through the spindle fibers; for instance, the accessory in individual number 45 (plate 7) has numerous fibers from both poles attached to it and its appearance certainly indicates that force is being exerted at the points of attachment.

It will be noted from the foregoing that any J-shaped tetrad has the capability of appearing in other individuals as a chromosome of either the Stenobothrus or Hippiscus type. Using this knowledge as a key, it is evident from a study of animals number 3 and number 32 (plates 2 and 5) that all of the chromosomes except number 2 and number 3 possess the potentiality of being of the Stenobothrus type in other individuals. If we then follow chromosomes number 2 and number 3 through the plates, we find chromosome number 3 in individual 16 (plate 3) to be a J, and in individual 21 (plate 4) we get the combination of two atelomitic homologues giving a Stenobothrus type of tetrad. Again, in individual number 31 (plate 5) it is a J. It is also of this form in animals number 7 (plate 2) and number 22 (plate 4). Chromosome number 2 does not occur except as a tetrad of the Hippiscus type in my collection. There was some fear that I might be confusing chromosomes number 3 and number 5 because of their similar size and because not more than one in any individual varied from the Hippiscus form until I reached individual number 31 (plate 5). Here all of the chromosomes are either of the Stenobothrus type or J's except numbers 1 and 2 and they are too small for any confusion. Number 1 is freely of either type, so that we should expect to find, if a sufficient number of animals were studied, some with ten tetrads of the Stenobothrus type. The nearest approach to this possibility in these sixty-two specimens is the occurrence of eight in individual number 6 (plate 2). On the other extreme, it has already been noted that three rings and the accessory are atelomitic throughout. This would leave a possibility of eight telomitic tetrads. Individual number 62 (plate 9) is of this composition.

Plates 2 to 5 present quite a different appearance from plates 6 to 9, for the former group has many more chromosomes with nonterminal fiber attachment than the latter. The results are recorded in the order in which the animals were collected, which was also the order in which they were studied. The individuals represented on plates 2 to 5 were taken in the Yosemite Valley; Sisson, Cal.; and Eugene, Ore. Those represented on plates 6 to 9 are from Friday Harbor and Olga, Wash.; La Grande, Ore.; and Pocatello, Ida. They grouped themselves as shown without the slightest rearrangement. I refer to the first group as form A and to the second as form B.

The following table, made up from the plates, gives the total number of atelomitic and J-shaped tetrads. The first and third columns represent form A and the second and fourth columns represent form B. Each horizontal row, therefore, represents two individuals. The first horizontal row represents individual number 2 (plate 2), form A, in columns 1 and 3, and individual number 33 (plate 6), form B, in columns 2 and 4. The successive horizontal rows represent successive individuals in the two forms, i.e., numbers 3 form A, and 34 form B in the second row, numbers 4 form A, and 35 form B, in the third row, etc.

The ratio of atelomitic to J-shaped tetrads in the case of form A is 0.42; in the case of form B, 0.47. One might have expected in place of this relatively constant relation that the larger num-

NUMBER ATELOMITIC TETRADS	NUMBER ATELOMITIC TETRADS	NUMBER OF J TETRADS	NUMBER OF J TETRADS
Form A	*Form B*	*Form A*	*Form B*
5	4	1	1
6	4	3	2
5	4	4	2
5	3	3	1
8	3	0	1
4	4	5	1
5	3	2	2
7	3	2	2
5	4	2	1
6	4	1	1
4	3	4	2
5	4	1	2
4	4	4	2
6	4	1	1
6	5	2	2
6	3	2	4
6	4	3	2
3	3	5	1
6	3	2	2
7	4	1	3
7	4	2	2
5	3	3	1
5	4	3	2
4	4	3	1
6	3	1	3
6	4	2	1
3	3	4	2
6	3	1	5
7	4	1	1
6	3	3	6
5	3	4	1
Average....5.77	3.6	2.4	1.7

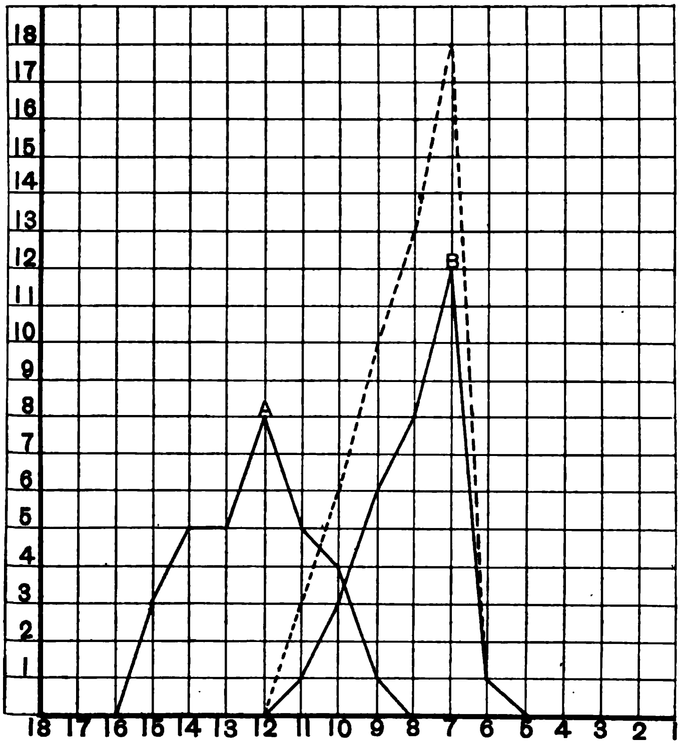
ber of Stenobothrus rings in form A would be compensated for by a smaller number of J's, or heteromorphic forms and the smaller number of Stenobothrus rings in form B by a larger number of J's. This might have indicated that one form was more viable in a given environment than the other. What we have is a uniform decrease in both Stenobothrus rings and J's in form B and a compensatory increase in the number of telomitic tetrads. The most important point to be noted is that if we may assume that heteromorphism is an indication of heterozygosity, then form

B is only about one-half as heterozygous as form A and consequently may be expected to be less variable taxonomically. That it really is less variable is indicated by the fact that taxonomists have separated the individuals that largely compose form A into four species, while they have treated the group that chiefly composes form B as a single species.

Since chromosome number 1 is the only tetrad subject to heteromorphism which could be recognized without possibility of confusion, its composition was studied in the two groups. Its behavior in the whole collection has been described on page 458. It occurs as follows:

	ATELOMITIC	J-SHAPED	TELOMITIC
Form A	8	16	7
Form B	6	16	9

In both cases it approximates closely a 1-2-1 ratio as it did for the entire collection. While it is evident then, that a rather general change has taken place in the two forms in respect to the number of atelomitic and telomitic chromosomes, it has not affected chromosome number 1. Likewise, a glance through the plates will show that the accessory (no. 4) and chromosome number 2 have remained constant. The remaining nine tetrads intergrade in size so closely that it is impossible to identify any given one with certainty. It was, therefore, necessary to study them collectively. The number of atelomitic dyads per individual was determined and the results plotted. The curves thus derived are shown in text figure 1. Numbers 10, 11 and 12 have remained constantly atelomitic, if I have been correct in my identification of them. But on the chance that I may not have been able to identify these correctly I shall include them in the curves; if their form is constant, they will not affect the shape of the graphs, while, on the other hand, there is a possibility that they are concerned in the changes. In text figure 1 the ordinates represent the number of animals; the abscissae the number of atelomitic dyads per cell. That is, each Stenobothrus ring is made up of a pair of atelomitic dyads, while the J-shaped tetrads have only one. For example, individual number 3 has five Stenobothrus rings and three J-shaped tetrads making thirteen V-shaped dyads derived from the tetrads in question—that is, not including numbers 4, 2 or 1. We have then in these nine (fig. 1) tetrads, which



Text-fig. 1 Curves showing the number of atelomitic dyads, exclusive of chromosomes number 1, 2 and 4. The abscissae represent the number of such dyads, the ordinates the number of individuals, the broken curve includes data from twenty additional animals of form 'B.'

are concerned in the changes, the possibility of eighteen atelomitic dyads. The two solid curves each represent the same number of individuals, thirty-one, the total number in the collection of form 'A'. There were twenty more animals of form 'B' which have been studied but not included in the plates, since they show no exceptional conditions. Data from these twenty are used in the broken curve of form B, which includes all of the specimens of this form in my collection.

Analysing these curves we see that in form A the extreme numbers of atelomitic dyads are 15 and 9, the mode 12 and the mean 12.3. In form B the extremes are 11 and 6, mode 7 and mean 8. It will be noted that the extremes in neither group reach to the mode of the other. The graph of form A is well balanced, indicating a stable group with considerable range of variation. That of form B, while showing much less variation, is skewed to the left, which would lead one to suspect that this group is changing towards the more stable form.

Plates 6 to 9 show chromosomes number 3 and number 5 always of the Hippiscus type in form B, though both are subject to change in form A. There is some question as to whether chromosome number 5 is always correctly identified. However, it is clear from animal number 60, which has three rings of the Stenobothrus type and five J-shaped tetrads, that eight tetrads have the potentiality of being atelomitic. It is puzzling that five (animal no. 47) is the nearest approach to this extreme found. Animal number 60 with its possibility for eight Stenobothrus rings offers one of the strongest indications that these two groups may be really members of a single species.

c. Segregation and recombination of homologues of J-shaped tetrads in eleven individuals of Circotettix lobatus. Eleven specimens of this species were taken in a very circumscribed area at La Grande, Ore. and a single specimen of another species (rabula) at Ogden, Utah. This latter animal had both telomitic and atelomitic chromosomes, but no J-shaped tetrads. This is not significant, however, as three of the eleven individuals of lobatus also lack J-shaped tetrads.

First spermatocyte complexes of eight of the eleven lobatus are shown (plate 10). The other three are included in the description. As just stated, three (or over one-fourth) of these animals have no J-shaped tetrads. Four have one and the remaining four have two. As in Trimerotropis, their dyads segregate at random in regard to each other and to the accessory.

The minimum of atelomitic tetrads in this limited collection is four (animal no. 64, plate 10) and the maximum six (animals no. 69 and no. 71). Animal number 67 has, in addition to five telomitic tetrads, two J-shaped tetrads, exclusive of chromosome number 1, which in this instance is rod shaped, but which we see from other individuals may be atelomitic. In a large group of animals, then, one would expect to find a maximum of eight atelomitic tetrads. This species agrees in this respect with form 'B' described above. Chromosome number 1 is of especial interest; in four cases, it is of the Stenobothrus type, in three, rod-shaped, and in three, J-shaped, while in one animal (no. 70) it is extremely unequal. I will have more to say later concerning this point. Segregation and recombination of the dyads of the heteromorphic tetrads in this species does not differ from that in the larger group.

d. Origin of a tetrad with unequal dyads. Tetrad number 1, animal number 70, as mentioned above, divides very unequally as shown on plate 10. Furthermore, the fiber attachment is not terminal on the smaller part, which in size suggested at once that it was the free arm of the atelomitic dyad of the heteromorphic form of this tetrad. A study of the late prophases gave the key to the solution of the problem as to what might cause such an inequality. Here we find a tetrad, normal to all appearances, except that a pair of chromomere vesicles,[2] (so called plasmasomes) described by the writer in 1913, is attached near the middle of one dyad (fig. 70*d*, plate 11). Wenrich ('16) identified these vesicles as expanded conditions of certain granules, terminal in the case of his tetrads 'A' and 'B'. We also know from from the work of Pinney ('09) that the point of fiber attachment is usually marked by prominent granules. It, therefore, seems probable, especially since the other end lacks them, that the vesicles mark the point of fiber attachment on what should have been a J-shaped tetrad and that their formation has weakened the dyad with which they are associated to such an extent that division in metaphase occurs at this point. The result is that one and a half dyads go to one pole, the remaining half dyad to the other. This peculiarity seems to be constant for this animal. Figure 38*a* (plate 11) represents a similar possible origin for an unequal pair in one of the larger chromosomes in form B. This was the only instance of the kind found in this animal.

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[2] It seems desirable to qualify the term vesicle which I used ('13) to designate these structures as chromomere vesicles or vesicular chromomeres, as the case may be, in order to prevent confusion with the vesicular chromosome or chromosomic vesicles first described for the Orthoptera by Sutton ('00).

The suggestion comes that they may stand in somewhat the same relation to certain chromomeres as the chromosomic vesicles do to the chromosomes. Just as given chromosomes (e.g., the accessory) have a more marked tendency than others to become vesicular, these specialized chromomeres of different chromosomes may vary in this power. For example a chromomere, or granule, may merely become expanded with the chromatin more or less equally distributed as Wenrich ('16) has shown for one of the terminal granules of tetrad 'A' of Phrynotettix. Such an expanded chromomere may or may not go a step further and form a definite membrane giving a true vesicle; e.g., one of the terminal chromomeres of tetrad 'B' in Phrynotettix.

e. Other forms of heteromorphism. Two other types of heteromorphism remain to be considered. The first—a peculiarity common to both these genera—is a constriction of certain dyads of chromosomes number 3 and number 5. The behavior of tetrad number 5 in different individuals is shown in plate 14. Figures 21*a* and 13*a* are homomorphic, constricted and smooth forms, respectively. Figure 62*f* is the heteromorphic form. The same thing is shown in chromosome number 3 (plate 2), animal number 6 being homomorphic for the constricted form, number 2 homomorphic for the smooth rod and number 8 heteromorphic. Tetrads marked by this peculiarity are almost as striking as the J-shaped tetrads. And since chromosome number 5, frequently, and number 3, occasionally, are J-shaped or even of the Stenobothrus type, the J-shaped tetrads may have the telomitic dyads of the constricted form (plate 3, 16, chromosome no. 3); or of the usual smooth rod form. In other words, for these two chromosomes, there are three types of homologues: atelomitic, telomitic-smooth and telomitic-constricted.

The third type of heteromorphism affects chromosomes number 9 and number 11 as they are arranged on the plates. Both homologues are atelomitic, but there has been a secondary shifting of the point of fiber attachment so that the free arm of one dyad is much longer than the free arm of the other. The point of union of the homologues is correspondingly nearer the bend in the dyad with the long free arm. Photomicrographs of these conditions are shown, figures 22*c* and 22*d* (plate 14). As indicated by the numbers, these two chromosomes occur in the same individual (plate 4, 22). They are not modifications of the same chromosome, for in numerous instances both are present in a single section of a cell. Figure 23*a*, from another specimen, shows a tetrad which is homomorphic for the long-armed condition of the homologous chromosome shown in figure 22*c*, while figure 10*a* is probably the homomorphic short-armed form of the same chromosome from another specimen. At least, this latter individual is homomorphic for the short-arms as may be seen from the drawing of the entire complex (plate 3, 10, chromosome no. 11). The homomorphic forms of tetrad number 9, shown in 22*d*, are represented in figures 17*a* and 10*b* (plate 14). Chromosome number 9 may also be J-shaped (plate 2, 7) or telomitic (plate 4, 25).

All possible combinations of the dyads in these two types of heteromorphic tetrads occur and segregate freely in relation to sex.

In the two heteromorphic types described above, as in the case of chromosome 'C' in Phrynotettix (Wenrich '16), we have a visible mechanism whose behavior in the maturation divisions corresponds to the segregation of triple allelomorphs.

f. Reduced number of chromosomes in Circotettix. Circotettix is the only genus of the Oedipodinae, so far investigated, to have less than the typical number of chromosomes. Numerous counts showed eleven to be the number in the first spermatocyte and twenty-one in the spermatogonia (plate 11, figs. 70*a* and 75). I am not satisfied as to whether the missing chromosome is number 2 or number 3. Plate 10 is arranged to emphasize its absence. The figures are from Circotettix lobatus, but rabula agrees in this respect. It is idle at present to speculate as to whether the chromatin which would have gone to form the missing tetrad has disappeared from the complex or whether it has united with some other tetrad to form an octad such as Robertson ('16) postulates to account for the reduced number in

Stenobothrus. This latter genus has but nine chromosomes in the reduced series (seventeen in spermatogonia). Three of these, however, are rings which present a typically different appearance from the remaining five tetrads. The basis of this difference is that they have entered the spindle extended parallel to its axis and have nonterminal fiber attachment. Robertson holds that these three chromosomes are really multiples; their point of union being represented by the position of the spindle fiber attachment. This would give twelve (the number typical for the Acrididae) as the real number of chromosomes in the haploid series. Woolsey ('15) has clearly shown such a linkage of chromosomes to be correlated with a reduction in number in several species of a Locustid genus. Furthermore, McClung ('16) has found conditions similar to those discribed by Miss Woolsey among the individuals of a single species of Hesperotettix.

The difficulty is, that Robertson puts forward a hypothesis that nearly all rings of the Stenobothrus type are multiples and are correlated with a reduction in the number of chromosomes. This is clearly not the case as regards either Trimerotropis (forms A and B) or Circotettix, for both have numerous tetrads of the Stenobothrus type and Trimerotropis has the normal number of chromosomes (twelve) while Circotettix has but one less. The number of rings of the Stenobothrus type varies from individual to individual, but the number of chromosomes is constant. In addition, the transition stage (J-shaped tetrad) between chromosomes of the Stenobothrus type and those of the Hippiscus type is present in these species. A further argument against the above hypothesis is the secondary shifting of the point of fiber attachment on certain chromosomes (figs. 22*c* and 22*d*, plate 14).

It is clear that we have two fundamentally different types of rings which are so similar morphologically that unless we get transition stages, as Woolsey ('15) has in Jamaicana and McClung ('16) in Hesperotettix, we have no criterion by which to separate them. Both enter the metaphase extended parallel with the axis of the spindle, both have non-terminal spindle fiber attachment, both give double V's in anaphase and both may occur where there is reduction in number without all of them being correlated with the reduction (Circotettix and the Stenobothrus-like form described by McClung, '14). Yet structurally one is an octad the other a tetrad. The octad has true terminal spindle fiber attachment, so far as the tetrads composing it are concerned, while the ring of the other type has real nonterminal spindle fiber attachment.

g. Supernumeraries. Another variation which has attracted attention in our collection of Acrididian material for the first time and which is relatively very abundant in the specimens collected during the summer of 1915, is the presence in certain individuals of one, or sometimes two, entities which I shall designate as supernumeraries. They possess the staining capacity of chromatin. In the spermatogonia they enter the spindle at metaphase and divide like any chromosome (fig. 62*a*, chromosome *s*, plate 11 shows this body in the spermatogonia). In the plates of the first spermatocytes these bodies have been placed to the left of chromosome number 12, and a glance through plates 2 to 10 will show their frequency of occurrence. Individual number 10 (plate 2) of form 'A' possessed two supernumerary chromosomes, one more than twice the size of the other. This was the only animal in this group which possessed supernumeraries. But when we pass to form 'B' (plates 6-9) we find them in eight out of thirty-one individuals, that is, in more than one-fourth of all the animals studied.

I would especially call attention to the similarity between the supernumerary and accessory in specimen number 62.

The suprenumeraries are also present in two of the eleven Circotettix studied. It is evident from the plates that these elements vary greatly in size; when two are present in a given individual there may be very little resemblance between them. At the first spermatocyte division they pass to one pole undivided. When two are present they segregate freely in relation to the accessory and to each other. At the second spermatocyte division they again enter the spindle, at least usually, and divide as in the spermatogonia (plate 13, 62*e*).

2. *Spermatogonial metaphases and female somatic metaphases*

a. Individual number 1. Dr. McClung ('14) has shown that generally the point of spindle fiber attachment is constant in all generations (spermatogonia, first and second spermatocytes). Therefore, knowing the number of dyads in the first spermatocyte with nonterminal fiber attachment, one would expect to find the same number in the spermatogonia. The animal represented on plate 1 has four Stenobothrus rings, each made up of a pair of dyads with nonterminal fiber attachment or eight such dyads in all; in addition, there are the atelomitic dyads of the three J-shaped tetrads and the accessory; so that we should expect twelve atelomitic chromosomes in the spermatogonia of this animal. Figure 1, plate 11, a spermatogonial complex from this specimen, shows the twelve atelomitic chromosomes. Entire complexes of spermatogonia were drawn in order to be certain that all of the chromosomes with nonterminal fiber attachment might be present. The telomitic chromosomes appear in outline only, as I wish to make the enumeration of the others as easy as possible.

b. Extremes found in the group. In order to show clearly the correspondence between the spermatogonial chromosomes and those of the first spermatocytes, two spermatogonial complexes, one from Form 'A', the other from Form 'B', have been rearranged so that the homologues are paired as they are in the first spermatocyte anaphase (with which they are compared) from the same individual. One of these spermatogonial complexes is shown with the chromosomes in their normal position in figure 32*a* (plate 11). The rearrangement of this complex may be seen on plate 5, figure 32*a*. Fifteen atelomitic dyads are present in both spermatogonium and first spermatocyte. Figures 36 and 63*a'* (plate 9) show similar arrangements of first spermatocyte anaphase and spermatogonial complex, respectively. Each has eight atelomitic chromosomes. Figure 63*a'* is a rearrangement of 63*a* (plate 11).

From the analysis of the first spermatocyte metaphases, we would expect animal number 62 to have the least number of atelomitic spermatogonial chromosomes, seven. This is seen to be the case in figure 62*a* (plate 11). Information derived from the same source would lead us to expect seventeen atelomitic chromosomes in individual number 22. Such a metaphase is shown on plate 11, figure 22*a*. Figures 62*a* and 62*b* are spermatogonial metaphases from the same animal and have seven atelomitic chromosomes, as we would expect from the first spermatocyte complex of this individual (plate 9, 62).

Figures 70*a* and 75 (plate 11) are spermatogonial complexes from Circotettix lobatus. The significant difference from similar complexes of Trimerotropis is that they have twenty-one instead of twenty-three chromosomes. In regard to the atelomitic members the conditions are the same as in Trimerotropis, that is, the number of such dyads is the same in both spermatogonial and first spermatocyte metaphases. The number is twelve in these two cases. Figure 70*a* is from the individual in which tetrad number 1 divides very unequally in the first spermatocyte. It will be seen from this figure that the smallest two homologues are equal in size but one has median a fiber attachment. This agrees with the expectations from the study of the prophases of the first spermatocyte which showed a normal tetrad. We have then in the tetrad which divides unequally in the first spermatocyte, in reality a J-shaped tetrad which is dividing at the point of fiber attachment in the atelomitic dyad.

c. Female somatic complexes. Unfortunately, the maturation stages in the female are inaccessible because they do not take place until a few hours before or even after the egg is deposited (Henking '91), and the great quantity of yolk present has so far defied all efforts at sectioning. Immature ovaries are readily sectioned and contain numerous divisions in the follicular cells. These have been used for the somatic complexes. Morrill ('10) found from a study of the cleavage and early blastoderm stages of some Coreid Hemiptera that the number and size relations of the chromosomes in the somatic cells of the males and females are the same as in the spermatogonia and oogonia, respectively. Three females were studied by me, the complex proving constant for the individual, but varying from animal to animal. Specimen number 74 with its fourteen atelomitic chromosomes (plate 11, 74) was taken in the Yosemite Valley, hence should belong to the group having the larger number. The other two are from Puget Sound, where we would expect the smaller number. One has ten (plate 11, 72) and the other, much to my surprise, has eight (plate 11, 73), which, if my conclusion that three of the atelomitic rings are constant is correct, would be the minimum number for the female, since she possesses two accessories, giving a complex of twenty-four chromosomes instead of twenty-three as in the male.

We may now answer the third and forth questions propounded at the beginning of this paper. The complex is constant for any given individual but varies within fixed limits for the species. Hence there is no need to assume any regulatory mechanism during maturation or fertilization.

3. Second spermatocytes

a. Types found in individual number 1. As has already been noted, the formula, 2^n, in which *n* represents the number of pairs of chromosomes under consideration, gives the possible combinations in the gametes. According to this formula animal number 1, as we have seen on page 455, would be expected to form sixteen sorts of second spermatocytes. However, as we can not distinguish between chromosomes 7 and 8, we can identify only twelve sorts, as shown on plate 12. Numerically, they fall into two classes, those with eleven (figs. 1 *j* to *o*) and those with twelve chromosomes (figs. *p* to *u*), owing to the presence or absence of the accessory. In the class with eleven chromosomes we would always have the four atelomitic chromosomes derived from the four Stenobothrus rings (fig. 1 *j*). In addition we may have one of the larger atelomitic dyads (fig. *k*), the small atelomitic dyad (fig. *l*), the small atelomitic dyad and one of the large ones (fig. *m*), both large ones without the small (fig. *n*), or the atelomitic dyads of all three (fig. *o*). Figures *p* to *u* present a similar series, except that they each have one more atelomitic dyad, the accessory.

b. Extremes found in the group. Two individuals, number 6 (plate 2) and number 62 (plate 9), have no J-shaped tetrads and therefore would have only the two recognizably different sorts of second spermatocytes determined by the presence or absence of the accessory. Number 7 (plate 2) and 60 (plate 9), on the other hand, each have five J-shaped tetrads which, in combination with the accessory, would give 2^6 or sixty-four different sorts of spermatozoa. The distribution of the J-shaped tetrads in forms 'A' and 'B' as well as in the whole group, is shown by the curves (text-fig. 2). The ordinates represent number of individuals, the abscissae the classes of spermatozoa formed. The lower broken curve represents form 'A', the solid line form 'B', while the upper broken line is a composite of the two. It is interesting to note that while the extremes of both forms are the same and contain the same number of animals, there are only four of the thirty-one members of form 'B' which give more than eight kinds of spermatozoa, while fifteen, or practically half, of the members of form 'A' give sixteen or more kinds.

Individual number 62 is of interest on account of the large supernumerary present in the spermatogonia and first spermatocytes. There are no J-shaped tetrads in this specimen, but it is possible to recognize four sorts of second spermatocytes. Of two sorts with three atelomitic dyads one has eleven chromosomes and the other twelve—due to the presence of the supernumerary. Two sorts have four atelomitic dyads, i. e., the accessory in addition to the three derived from the three Stenobothrus rings of the first spermatocyte. One of these has the normal number (twelve) and the other thirteen, since the latter also contains the supernumerary.

Individual number 63 (plate 9) is one of the easiest to follow out, since it has only one J-shaped tetrad. This, with the accessory, gives two differential chromosomes, from the distribution of which we should expect 2^2 or four sorts of second spermatocytes. Considering all the atelomitic dyads, including those derived from the three Stenobothrus rings, we find four morphologically different sorts of second spermatocytes as follows:

11 with 3 atelomitic dyads (fig. 63*d*)
11 with 4 atelomitic dyads (fig. 63*c*)
12 with 4 atelomitic dyads (fig. 63*b*)
12 with 5 atelomitic dyads (fig. 63*e*)

The transformation stages which result in the mature spermatozoa appear to be perfectly normal throughout the collection.

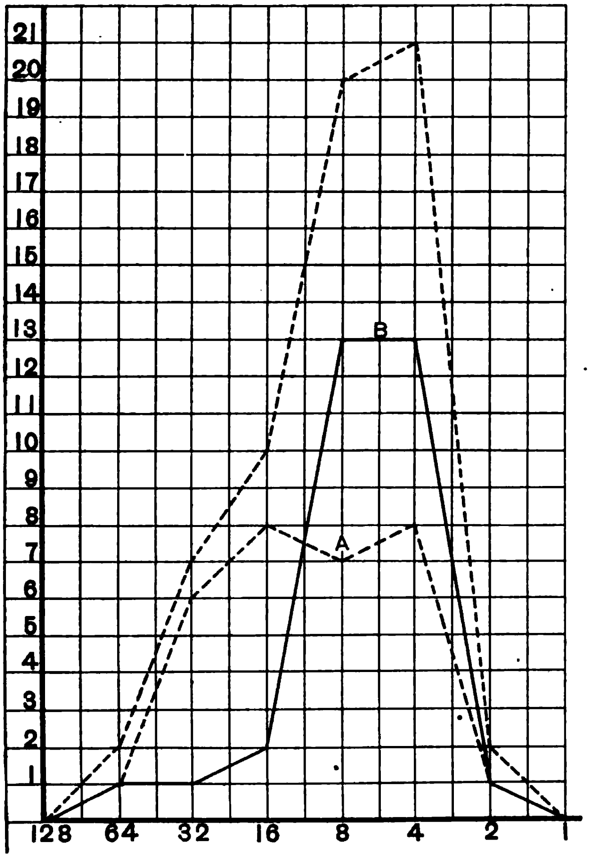
c. Circotettix. Circotettix does not differ from the species just described so far as the formation of different types of spermatids is concerned. But we have the important difference that the numbers are ten and eleven (barring the supernumeraries which are present in two of the specimens) instead of the usual eleven and twelve. Complexes from two individuals are shown in figures 69*a*, 69*b* and 70*b*, 70*c* (plate 13).

III. SUMMARY OF OBSERVATIONS

1. The number of spermatogonial chromosomes in Trimerotropis is twenty-three. Anywhere from seven to seventeen of these have been found to be atelomitic, but the number of such chromosomes is constant in a given individual.
2. The number of spermatogonial chromosomes in Circotettix is twenty-one. Nine to thirteen of these have been found to be atelomitic in different individuals. The number of atelomitic chromosomes is constant for the individual.
3. The number of somatic chromosomes in the female of Trimerotropis is twenty-four. The number of atelomitic chromosomes varies in different individuals from eight to fifteen, but is constant for the individual.
4. The number of chromosomes in the first spermatocyte of Trimerotropis fallax is twelve, of Circotettix eleven.
5. Certain individuals may possess one or two supernumerary chromosomes. These bodies divide in the spermatogonia, pass to one pole undivided in the first spermatocyte division and divide in the second spermatocyte. They segregate in the

first spermatocyte without relation to the accessory, or to each other when two are present.

6. The first spermatocyte tetrads show three types of heteromorphism: 1) one homologue telomitic, the other atelomitic,



Text-fig. 2 Curves showing the number of morphologically different classes of spermatozoa. The abscissae represent number of classes, the ordinates number of individuals. The large broken line is a composite of 'A' and 'B.'

giving J-shaped tetrads. 2) both homologues atelomitic, one with nearly median, the other with subterminal fiber attachment. 3) both homologues telomitic, one smooth, the other constricted; or, one telomitic constricted, the other atelomitic.

7. All of these types of heteromorphic homologues segregate at random in the first maturation division.

8. Considering the J-shaped tetrads alone, of sixty-two males, two formed sixty-four morphologically different sorts of spermatozoa, seven formed thirty-two sorts, ten formed sixteen, twenty formed eight, twenty-one formed four and only two were dimorphic.

9. The complex is constant in the individual for somatic, spermatogonial and first spermatocyte metaphases and has a fixed range of variation for the second spermatocytes. Certain exceptions to this general rule have been noted in the text, e.g., the accessory with numerous fibers attached (plate 7, 45) and the tetrad which divides unequally in Circotettix (plate 10, 70).

10. The complex varies widely in the group within the limits of the above mentioned types of heteromorphism, indicating, so far as my material goes, that random mating of the various classes of morphologically different gametes has occurred.

11. Taxonomically the group is exceedingly variable.

IV. DISCUSSION

1. Constancy in individuals

To me one of the most impressive facts in this whole work has been the very great degree of individual constancy of the complex which has been preserved in the organism in spite of the wide range of morphological variation between the homologous chromosomes in these species. It should be emphasized that the term individual constancy is applicable here, although it is used in a broader sense than is ordinarily the case; that is, while the somatic, spermatogonial and first spermatocyte complexes are practically identical for the respective generations in a given individual, there is a fixed number of morphologically different classes of second spermatocytes for each individual, but in all cases these can be calculated from a knowledge of the first spermatocyte complex.

It is futile to speculate at present on the cause of this heterogeneity, but so far as the doctrine of chromosome individuality is concerned the manner of its origin is of minor importance. The essential thing is that it exists, and is transmitted, not only from cell generation to cell generation in the individual, but occurs so definitely that one can predict the combinations that should occur in the population. Judging from the conditions present in my material one would find all possible associations if a reasonably large number of specimens were examined.

Actual observation has shown the ratios of the telomitic to the atelomitic dyads of chromosome number 1 to be 1 to 1. In other words the variant, atelomitic homologue, occurs in practically one-half of the population. But we cannot generalize that this ratio holds, therefore, for all of the variants, if we may so designate all atelomitic dyads. Tetrad number three has only six atelomitic dyads out of a possible one hundred and twenty-four in the sixty-two individuals represented (plates 2 to 9). I is impossible to calculate ratios for the occurrence of any given combination in the general population unless one knows in advance the frequency of occurrence of that particular variation.

It is hard to imagine a better example of persistent individuality than that illustrated in figures 21*a*, 62*f*, 13*a*, plate 14 where a variation from the normal smooth rod form (fig. 13*a*), such as the constriction of the dyads (fig. 21*a*), has been transmitted, not only from cell to cell in one individual, but apparently on to the next generation, so that the union of a gamete carrying such a chromosome with one bearing its normal homologue has resulted in a zygote which shows both types clearly(fig. 62*f*). This is the same principle found throughout this work where no kind of heteromorphism (J-shaped tetrads and atelomitic tetrads with unequal arms) has been found without homomorphic forms corresponding to both types of dyads occurring in other individuals.

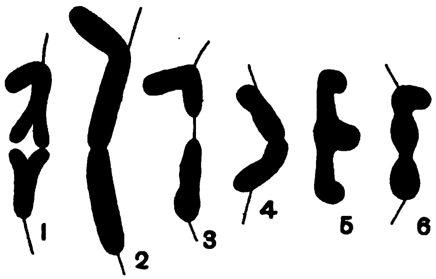
There are but two other instances in all cytological literature so far as I know where homologous chromosomes show morphological differences other than those of size. One of these is the multiple of Mermeria (McClung '05). The other case is that reported by Voïnov ('14). It seems desirable to undertake a rather extensive review of the latter paper on account of some questionable conclusions and on account of some points of general similarity, as I believe, in our material.

Voïnov is dealing with Gryllotalpa vulgaris. This is the form for which vom Rath ('92) reported the somatic number of chromosomes as twelve. He described a doubling of this number just before the first maturation division which reëstablished the normal number. The second division brought about reduction to one-half the normal. Vom Rath extended to all animals his conclusion that there is a doubling of the number of chromosomes before the maturation divisions, both of which are reductional and result in halving the normal number of chromosomes. As this erroneous idea appealed to many as a support of Weismann's hypothesis it was widely accepted for a time. Hence work on this form is of more than usual interest.

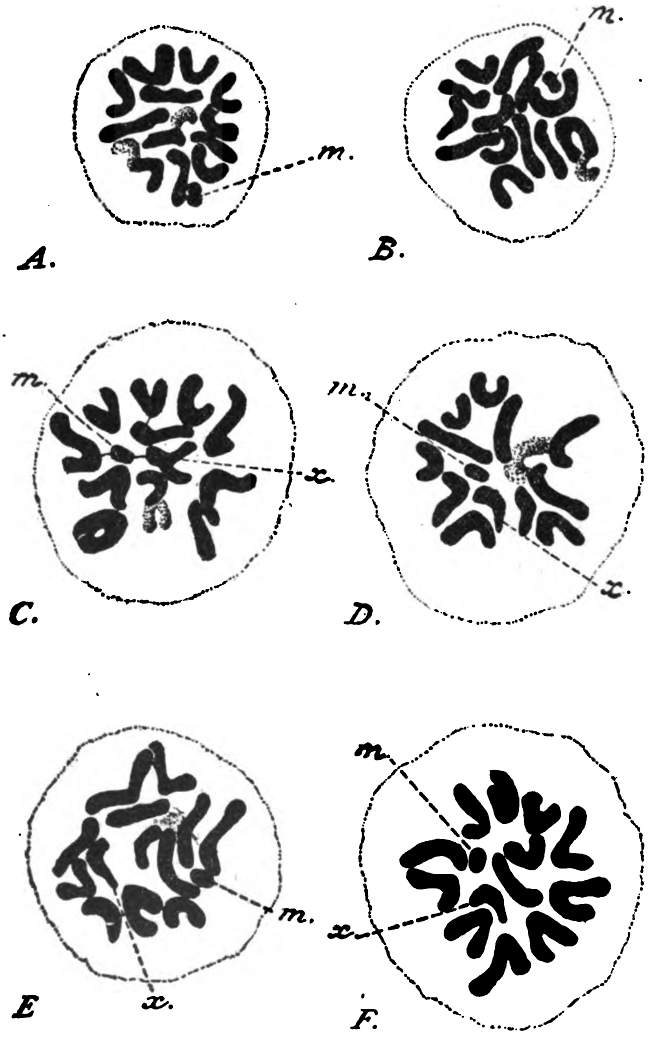
Voïnov figures fifteen chromosomes in the spermatogonia. The smallest of these is clearly without a morphological mate and he considers it to be in reality a pair of microchromosomes synapsed in the spermatogonia. This assumption leaves him without a *Y* for what he considers an *XY* pair which occurs in the first spermatocytes. He assumes the *Y* exists in the spermatogonia but has escaped detection. Therefore, he concludes that seventeen is really the somatic number, while he finds seven in both maturation divisions. This is accounted for by further assuming that two of the seven must be multiples; one an octad, the other a hexad. The hexad he believes to be identical in composition with the chromosome 'en L' of de Sinéty ('01) and the hexad of McClung ('05). A comparison of Voïnov's chromosome marked 'a' and 'bi' (text fig. 8*c*, see my text fig. 3, *3*) with de Sinéty's chromosome *c–s* (fig. 93, plate 3, see my text fig. 3, *1*) and with McClung's chromosome 'ac' (text fig. 3, see my text fig. 3, *2*) will show a critical difference. In both the latter, if we may assume that de Sinéty's chromosome 'en L' is a hexad, the tetrad part divides, resulting in a greater quantity of chromatin passing to one pole than the other, while Voïnov's 'hexad' divides in such a manner that, though the parts are morphologically different, the quantity of chromatin in each would seem to be about the same. Voïnov recognizes this difference, he says (p. 474):

> Il s'ensuit que le chromosome accessoire est distribué seul à un pôle et le bivalent entier avec lequel il était associé va au pôle opposé.
> Ce bivalent donc, comme le chromosome accessoire, ne se divise pas dans la première mitose de maturation. Cette manière de se comporter constitue une différence avec les chromosomes multiples décrits par Sinéty et Clung.

That is, while its structure is the same, its behavior is different. It will be obvious to the reader that this chromosome corresponds both in form and in behavior in the individual with the J-shaped tetrads described in this paper. But, if it does correspond, one would expect certain individuals to contain in its place a rod-shaped tetrad and others a ring of the Stenobothrus type. That such a ring occurs in Voïnov's material may be seen in his figure 32*t*, plate 24, or my text figure 3, *5*. While he has material from fifty animals, it is impossible to judge how thoroughly it has been studied from the standpoint of the occurrence or non-occurrence of this 'hexad.' The only direct statement is in regard to a count to show the distribution of its parts in relation to those of an unequal pair. This

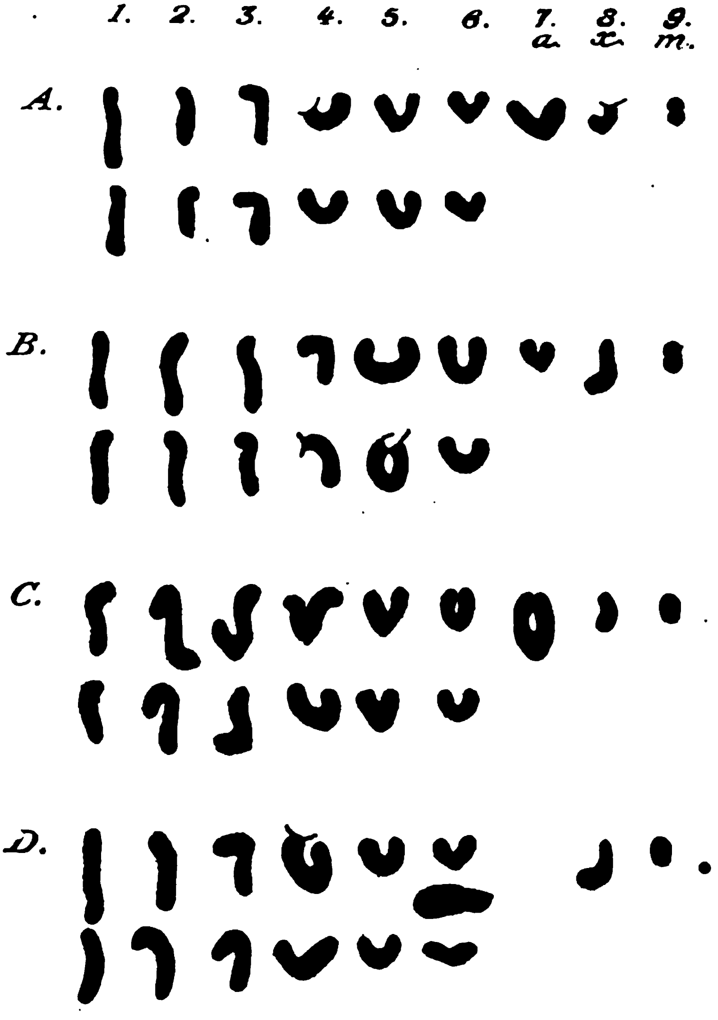


Text-fig. 3 1 'Chromosome en L,' de Sinéty, plate III, figure 93.
2 Hexad multiple, McClung, '05, p. 309, figure 3.
3 'Chromosome -L,' Voïnov, '14, p. 473, figure 8*c*.
4 Late second spermatocyte metaphase of 'l'element bivalent' Voïnov, p. 488, figure 16*c*.
5 'La grande tétrade,' Voïnov, plate XXIV, figure 32*t*.
6 'L'idiochromosome, XY,' Voïnov, Plate XXIV, figure 29.



Text-fig. 4 A photographic reproduction of Voïnov's text-figure 1, showing that different individuals possess different numbers of V-shaped (atelomitic) chromosomes. A and B from the same individual show eight or nine. C from a different animal at least eleven, possibly thirteen. D from a third animal nine or ten. E and F from a fourth specimen have eleven.

count, he states, is based on a single individual. However, if my interpretation is correct, one should find varying numbers of atelomitic chromosomes in the spermatogonia of different individuals, while on Voĭnov's statement the complex should



Text-fig. 5 A photographic reproduction of Voĭnov's text-figure 2. The difference in the number of telomitic and atelomitic chromosomes possessed by individuals B and C is well shown.

be morphologically the same for all individuals. Voïnov shows (p. 448, fig. 1, see my text fig. 4) spermatogonial complexes from four individuals. *A* and *B* are from the same individual and agree in having eight or nine atelomitic chromosomes. *D*, from another individual, seems to be of the same type, while *C* and *F* from two other individuals, have eleven or twelve. This variation is made conspicuous by the arrangement in his text-figure 2 which shows strikingly the difference of the complex of individual *C* from that of *B*. However, Voïnov states (p. 450) that the form and size are constant for the species. To quote: "Dans les dessins où les chromosomes des quatre premières plaques sont groupés suivant leur resemblance (fig. 2), on voit que la forme et la grandeur sont constantes pour l'espéce." On the contrary, should one judge by these figures, (see my text-fig. 5) one would conclude that even the number was not constant since *D* lacks chromosome number 7. Also numbers 7A and 7B which purport to be homologous chromosomes,—drawn even from the same individual, —certainly bear no resemblance in size. This may better be attributed to the fault of the observer than to the material, as chromosome 7B would seem from the drawing to be much more homologous with 6A, and one member of 6B with 7A.

This evidence, together with the fact that none of his second spermatocyte figures show any indication of the presence of a tetrad [His chromosome 'b' (p. 488 *C*, see my text fig. 3, *4*) looks like any ordinary dyad and not a tetrad], has convinced me that his 'hexad' is a J-shaped tetrad. This would simplify matters, as on Voïnov's interpretation there are two sex-determining mechanisms present:—an accessory which forms part of the 'hexad' and in addition an unequal pair which he believes to be an *XY*. This leads him to remark (p. 496): "C'est un cas très rare, comme celui de Banasa calva parmi les Hémiptères, où de meme, grâce á la coexistence de deux chromosomes sexuels, naissent quatre catégories de spermatides, distinctes matériellement."

In 1905 Wilson reported the presence of an accessory and an idiochromosome pair in Banasa calva, but in 1907 he published a note (which apparently Voïnov has not seen) in which he identifies as a supernumerary the element that he had formerly reported as the accessory.

Payne found what he believes to be a sex group in Gryllotalpa borealis. That is, an accessory and an unequal pair, the larger member of which always moves to the same pole as the accessory.

McClung has recently been able, through a knowledge of the J-shaped tetrads, to solve the puzzle of the multiple in Mermeria where he reported ('05), p. 316 that, "Entire tetrads pass into the second spermatocytes." It is now evident that the accessory is associated with a J-shaped tetrad which divides in the usual manner. Since apparently the accessory always attaches to the telomitic dyad, there would seem to be formed a sex-linked association comparable in end results with Payne's interpretation of conditions in Gryllotalpa borealis, the difference being that a physical union is lacking in the latter case. Voïnov's figures of his *XY* pair (plate 24, fig. 29, see my text fig. 3, *6*) strongly suggest the association of a third element with a normal tetrad; making this a true hexad multiple. A study of Wilson's figures of idiochromosome pairs suggests that they also might be hexad multiples; i.e., that the *X* is the accessory plus one dyad of a tetrad of which the homologue is the *Y*. Since we know tetrads composed of unequal homologues exist, there is no reason apparent why the accessory might not be associated with either the larger or smaller member. This might account for instances in Drosophila, where the *Y* is larger than the *X*. This explanation of the known facts would seem simpler than that of Wilson ('11), who attempted to derive all from a primitive *X*-*Y* pair.

It may be well to note the bearing of the evidence as to the time of segregation brought out by this work on Bateson's 'Reduplication hypothesis.' Bateson ('15) in a study of the sweet pea found coupling of two pairs of factors which he represents by the formulae A a and B b, giving the heterozygote, A a B b. The zygotic ratios obtained in F_2 showed that certain combinations between these factors occur more frequently than others. This led Bateson to postulate the theory that the original zygotic cell divided into two similar cells, A a B b but that the second cleavage plane passed differently through these two cells so that one gave daughter cells A B and a b, the other A b and a B. Some of these four cells divided oftener than the rest so that more gametes of certain kinds were produced. As to the time of these segregation divisions he says (p. 299):

> Moreover the excess of gametes of parental composition characterizing the coupling- and repulsion-series must certainly mean that the position of the planes of division by which the four quadrants are constituted is determined with regard to the gametes taking part in fertilization—though the relative positions of the constituents of the cells may perhaps be maintained throughout the history of the tissues, it is easier to suppose that the original planes of embryonic division are determined according to those positions than that their influence can operate after complex somatic differentiation has been brought about.

Morgan ('15) has shown that the series of facts dealt with by Bateson is the same as those treated by Morgan and his students under the term linkage and that they are open to the same explanation.

It is evident that if such a somatic segregation as Bateson advocates occurred in Trimerotropis or Circotettix, we would have different types both of spermatogonia and of first spermatocytes in the individual, i.e. the metaphases would show varying numbers of atelomitic chromosomes, instead of being constant as I have found them to be.

2. *Heteromorphism, supernumeraries and reduction in the number of chromosomes*

The more I have studied this unusual group of grasshoppers the deeper has grown the impression that all of its peculiarities are closely interrelated. As was pointed out elsewhere (p. 465) there is some ground for associating the formation of vesicles with a shifting of the point of fiber attachment and also with a weakening of the chromosome at that point. In one animal, division constantly occurred at the point in question. It is conceivable that this weakness might result merely in a constriction such as marks certain homologues. Figure 38 *a* (plate 11), where one of the larger tetrads in a member of form B is dividing at such a point, favors this interpretation. This is the only instance of this sort found in animal number 38.

So far, in all of our work, we have never found one of the larger tetrads with unequal homologues. On the other hand such peculiarities occur rather frequently among the smaller ones. This may indicate that the smaller chromosomes are less important. That they may be in part dispensed with was shown by Wenrich ('16) who finds that his tetrad 'C' lacks both terminal granules in certain individuals; and it seems possible that chromosome number 2 has been entirely eliminated from Circotettix. The homologues of the larger tetrads may be so important that a zygote lacking in even a part of one cannot develop.

The further suggestion presents itself that irregularities in mitoses such as have just been described, may be responsible for the occurrence of supernumerary chromosomes. Gametes containing a complete dyad plus a portion of its homologue would probably be viable and one may readily imagine that the extra portion might become detached in a succeeding division and might then form a supernumerary. Such an element would probably contain merely a reduplication of factor loci already present in the two dyads from whose homologue it was derived and might therefore have no influence in heredity.[3] On the other hand a supernumerary might contain the locus for multiple allelomorphs, in which case it might have an influence. The varying sizes found are perhaps due to two causes: (1) origin from different tetrads, (2) degeneration. More than one-fourth of the population of form 'B' contain supernumeraries, and, though one would expect that they would automatically pile up, no individual has been found with more than two. It therefore seems evident that there must be some method of elimination.

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[3] Dr. P. W. Whiting has suggested that it may be of interest to geneticists to point out that chromosomes such as numbers 7 and 8 (plate 1), which are indistinguishable morphologically, behave as duplicates, corresponding to Shull's theory of duplicate genes, and not as tetraploid homologues, as Muller has suggested for Gregory's tetraploid Primulas, e.g. If A A' equals the atelomitic dyads and a a' their telomitic homologues on Shull's theory A would always segregate from a, and A' from a', or telomitic from atelomitic, giving a 1–2–1 gametic ratio. On the other hand, if they were tetraploid homologues in Muller's sense, A might segregate from A' and a from a' just as frequently, giving a 1-4-1 gametic ratio. In other words we would sometimes find the atelomitic dyad of chromosome number 7 mated with the corresponding one of number 8 and the same for the rod-shaped homologues. Since this condition does not occur, we must conclude that their morphological resemblance does not involve their behaving as tetraploids.

Wilson ('09) believes the supernumerary chromosomes in the Hemiptera are additional small idiochromosomes, and states that they are about the same size, of like behavior and show (Wilson, '07) some degree of coupling with the small idiochromosome. The supernumeraries found in the course of my work could not have had such an origin, since the sex-determining mechanism in the grasshopper is the accessory and not an X-Y pair. They are constant in size in a given individual, but range in different individuals from the size of the accessory (plate 9, 62) to less than a fourth of that size. In behavior during the growth period they simulate the accessory. If Wilson's hypothesis (that they are derived from the sex chromosome) were correct, my individual number 62, which appeared to be an entirely normal male, should have been a female; for the essential difference between the sexes is the possession of two accessories by the female, and in this particuar case the supernumerary, in the first spermatocyte metaphases simulates the accessory so closely both in size and appearance, that it is sometimes difficult to decide which is which. It seems probable that it was a large supernumerary of this type that Davis ('08) confused with the accessory when he described two 'monosomes' (accessories) as being present in one specimen of Arphia tenebrosa (p. 102).

3. Taxonomic variability and gametic composition of the group

My own slight knowledge of taxonomy, together with a recognition of similarity of habits and environment acquired through field work, enabled me to group these related forms as they were collected. The specimens were later turned over to

Mr. J. A. G. Rehn of the Academy of Natural Science for identification. My study of the germ cells was completed, the plates made and the curves plotted before I knew the result of Mr. Rehn's study. Similarly, Mr. Rehn classified the animals without knowledge of my results, or even of the localities from which the various specimens were taken. It is but fair to say that, owing to lack of time, all that Mr. Rehn attempted to do was to place the animals in the classification already established, although he hopes soon to undertake a revision of the group.

One of the most striking differences shown by the study of the germ cells is the apparent reduction in number of chromosomes in the form which is unquestionably Circotettix. There is no conflict here between taxonomic and cytological evidence. Two species come in this division, lobatus and rabula. Unfortunately, I have only a single member of the latter species and the present work has shown that any attempt to establish specific differences on one or two individuals is futile for this group.

The remaining eighty-three members of this group may be placed in two subdivisions, according to the number of atelomitic chromosomes in the duplex series as is shown in text figure 1 (page 463). The mode for one of these subdivisions is twelve, for the other seven. There is an overlapping of the two groups involving twenty individuals, ten from each form. But since the extremes of neither form reach to the mean of the other they might constitute two distinct species. However, since these groups are from widely separated localities, the differences may well be due to isolation. If we consider what the expected progeny of parents like number 60, form B, with its five J-shaped tetrads would be, it is evident that some of the offspring would be placed in form A according to this scheme.

It is interesting to note that all members of my form A were classified by Mr. Rehn as the Trimerotropis fallax of recent literature. On the other hand he placed three of the fifty-one members of my form B in this same species. The remaining forty-eight were identified as Circotettix suffusus as that species is at present recognized. He states in a note that this is a "Divergent Circotettix tending strongly towards Trimerotropis."

From my cytological studies, I do not hesitate to say that form B is a Trimerotropis, and furthermore, it is a question if Circotettix suffusus and Trimerotropis fallax, as they are now recognized, do not even constitute a single species.

4. Correlation of chromosomal behavior and Mendelian principles

The evidence pointing to the chromosomes as the bearers of the heredity determiners has been summarized so often recently that I shall not repeat the process. One of the chief difficulties that cytological research has met with has been the impossibility of distinguishing between the chromosomes derived from the two parents. While it has been clear that homologous chromosomes segregate into different gametes, it has been impossible to say, except in the cases of the tetrads composed of unequal dyads recently reported (Carothers '13, Wenrich '14, Robertson '15), that all of the chromosomes brought in by the egg do not pass into the female-producing spermatozoon, as was suggested by Payne ('09) as a result of his study of Gryllotalpa borealis.

Attempts have been made to determine the behavior of the paternal and maternal chromosomes in the maturation divisions and also to correlate given chromosomes with given somatic characters. Heretofore, the most promising line of attack on such problems has been through hybridization of forms with morphologically different chromosomes. The most noted of these experiments is that of Moenkhaus ('05). He crossed Fundulus heteroclitus and Menidia notata. The former has long, straight chromosomes, while those of the latter are short and curved. In the first few cleavages of the hybrid zygote, the chromosomes derived from one parent remained separate from those derived from the other. In later divisions the chromosomes became mixed on the spindle. But the important fact was demonstrated that the chromosomes derived from each parent maintained their morphological identity. The embryos did not develop beyond the closing of the blastopore.

Perhaps the most significant recent work along the line of hybridization is that of Federly ('13) who has combined breeding and cytology in his study of the moth, Pygaera. He crossed P. anachoreta and P. curtula, and found by a study of the spermatogenesis of the hybrid, that pairing occurs between only two chromosomes; thus in the first spermatocyte of the parent forms there are twenty-nine or thirty chromosomes, while in those of the hybrid there are forty-eight of about one-half the size of the parental forms. When a back cross was made with one of the parent species, normal pairing took place between about one-half of the chromosomes of the hybrid and those of the parent species to which the cross was made, giving about thirty large paired chromosomes among a corresponding number of small unpaired ones. As one would expect from this cytological knowledge, both the primary hybrids and those resulting from the back cross were intermediate in all characters except one or two which showed normal dominance and segregation. The difficulty in working with such a form lies in the large number of chromosomes and in the lack of any means of distinguishing between them.

The work of Morgan and his students on Drosophila ampelophila is too well known to need much discussion. Here there is the advantage of a small number of chromosomes which differ in size and shape. According to Metz ('14) there are four pairs as follows: a pair of microchromosomes, a pair of sex chromosomes, equal in the female (XX), unequal in the male (XY), and two large V-shaped pairs.

Breeding tests with numerous mutants have shown one large group of genes, over thirty in number, to be sex-linked, hence borne, presumably, in the X chromosome. Two other great groups of over twenty members each, segregate independently and are assumed to correspond to the two large euchromosomes. Two characters so far—bent wings reported by Muller ('14) and eyeless, by Hoge ('15)—have been found whose genes segregate independently of the other three groups. Should mutations occur with equal freedom at any locus in the chromatin it would be expected, as pointed out by Muller, that the small microchromosomes would show fewer mutations than the larger chromosomes, and since the genes for bent wings and eyeless must lie in one of the three pairs of euchromosomes, the presumption is that these two genes, rather than either of the two large groups of non-sex linked genes, lie in the microchromosomes. Should such prove to be the case, these would be the first instances of definite somatic characters being associated with a definite, recognizable euchromosome.

Dr. McClung, in two papers ('05 and '08), pointed out the general possibilities of cytological work and stated clearly his ultimate aim (p. 326, '05), "To determine the relation between individual chromosomes and characters in the body." For such a purpose the advantages of a species like Trimerotropis fallax or Circotettix lobatus is obvious. Taxonomically, there is striking individual variation, and correlated with this, apparently, are definite morphological variations, already described, of the homologous maternal and paternal chromosomes. Here, then, we have in a single species the means of distinguishing between certain homologous chromosomes. There is the further advantage of freedom from the sterility and lack of viability (if one may judge by the plentifulness of the animals) usually associated with hybrids. Of course it follows, that any ratios obtained through genetic work on these forms should be accurate, whereas those based on hybrids where there is a high degree of mortality, cannot be reliable.

So far no attempt has been made to breed these species, all of which, unfortunately for a worker located in the east, are Rocky Mountain forms. Another species of Circotettix occurs in mountainous regions of the east and, should it be as favorable cytological material as the western forms, I hope to do breeding work with it.

It might be said in passing that, according to my experience with several local species, grasshoppers are proving very favorable subjects for breeding. They stand captivity well and can accommodate themselves to various food and temperature conditions. They show strongly marked characters, breed freely, and it is possible to obtain three or four generations a year, at least of some species, by keeping them constantly at about 39°C. Special laboratory facilities, while convenient, are by no means essential since the eggs of most species normally live through the winter out of doors and even the adults endure great extremes of temperature. The prime requisites for this work, aside from the 'hoppers' themselves, are fine wire cages and patience.

In any case, a study of a collection of individuals living in their normal environment was desirable, for, had such conditions first been found in captive stock, one would surely have suspected that these unusual conditions were due to the artificial environment.

This work has been sufficient, I believe, to show the random segregation of the homologues of all but four (nos. 2, 10, 11 and 12) of the first spermatocyte tetrads, and that the recombinations found in the group correspond to what would be expected according to the law of chance.

V. CONCLUSION

This material clearly presents the following facts:

1. Constancy of the chromosome complex for the individual.
2. The occurrence of heteromorphic tetrads in the first spermatocyte.
3. The segregation of these heteromorphic homologues in the first maturation division according to the law of chance.
4. The formation of the expected classes of second spermatocytes.
5. Normal transformation stages for the spermatids.
6. The occurrence in a group of individuals of practically all possible combinations of the heteromorphic chromosomes.

These facts taken together may be accounted for by:

1. The ordinary process of free, chance fertilization acting in a species in which the present forms of the chromosomes which originated through some past reorganization are stable, or:
2. A reorganization of the complex, involving a change of fiber attachment for each individual before the setting off of the germ cells.

Both of these possibilities may be tested by breeding:—In the first case parents of known chromosomal constitution would give progeny with a fixed range of variation—limited by the number of possible combinations of the morphologically different gametes of the parents.

In the second case, any variation found in the group might occur in the offspring of a single pair.

The first explanation is a simple, logical one which meets all known facts; hence, in accordance with scientific custom, we are justified in accepting it for the present.

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PLATE 3

EXPLANATION OF FIGURES

Side views of eight first spermatocytes of form 'A' showing variation within the group.

10 Two supernumerary chromosomes, one large the other small.

SEGREGATION OF HOMOLOGOUS CHROMOSOMES PLATE 1

E. ELEANOR CAROTHERS

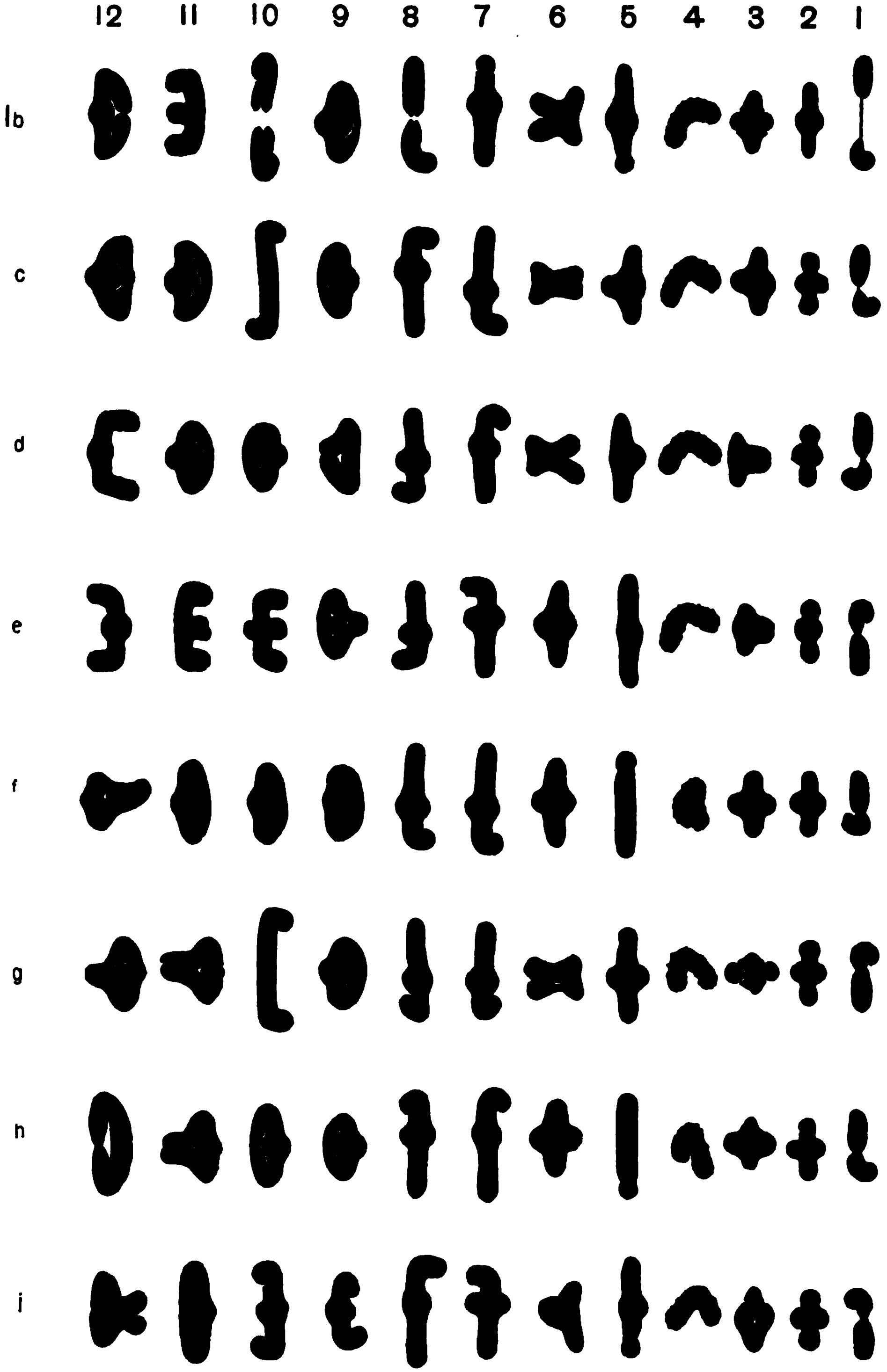


PLATE 7

EXPLANATION OF FIGURES

Side views of eight first spermatocyte metaphases, form 'B.'

41 Chromosome number 4 (accessory) broken.

43 Two supernumeraries, one very large.

45 Accessory (no. 4) with numerous fiber attachments.

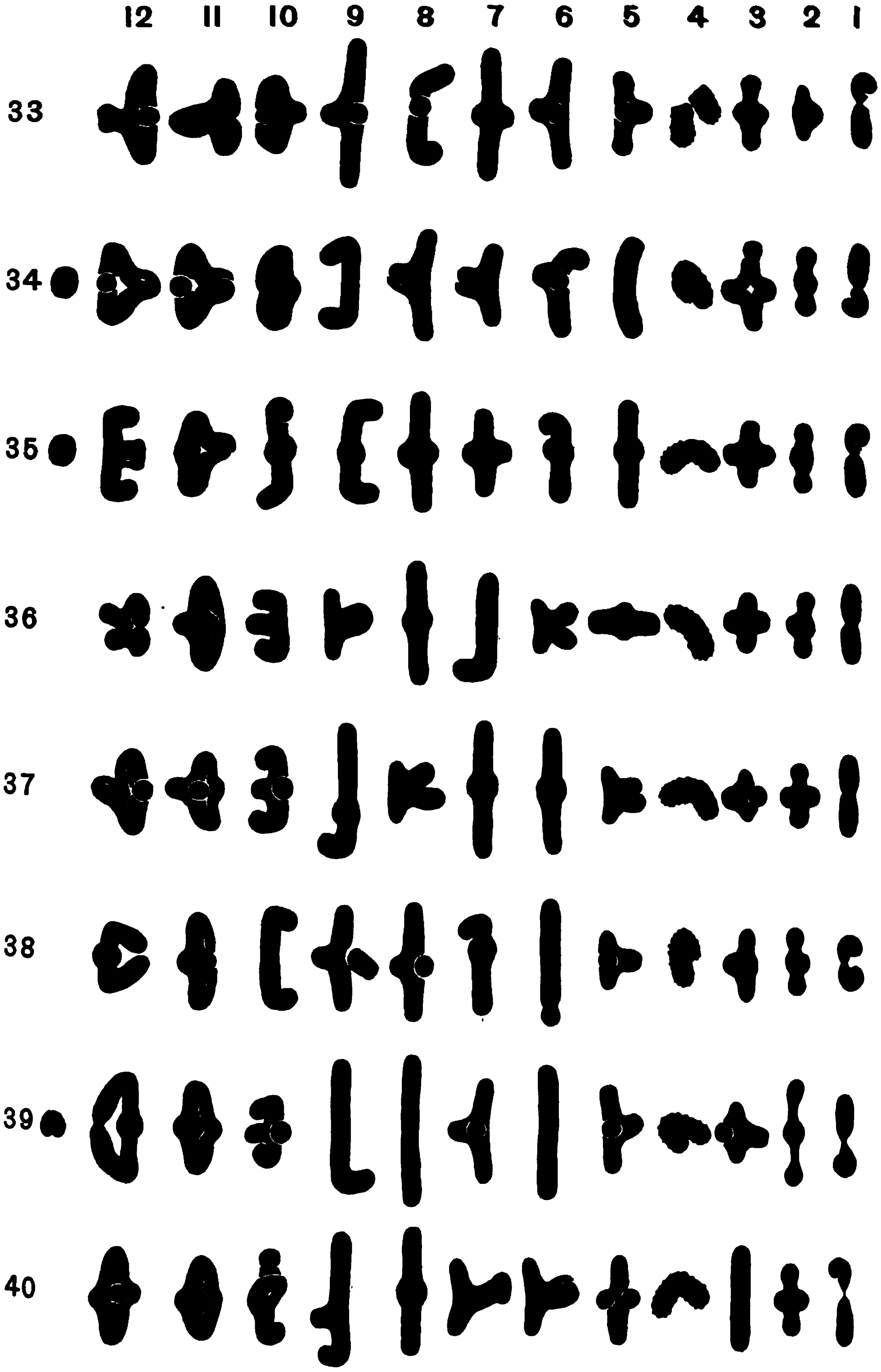


PLATE 9

EXPLANATION OF FIGURES

Side views of seven first spermatocyte metaphases, form 'B.'

62 This individual has the least number of atelomitic chromosomes found. Seven in the spermatogonia, resulting in three rings of the Stenobothrus type and the usual atelomitic accessory in the first spermatocyte. Note the very large supernumerary.

63 An early anaphase—chromosome number 4 cut.

63*a*′ Spermatogonial metaphase with chromosomes arranged in pairs. From same animal as 63. Note the very close resemblance to the first spermatocyte anaphase especially, chromosome number 8, one homologue of which is constricted in each, and chromosome number 7, one homologue of which is atelomitic in both cases.

For the four classes of second spermatocytes formed by this individual, see plate 13, 63*b* to *e*.

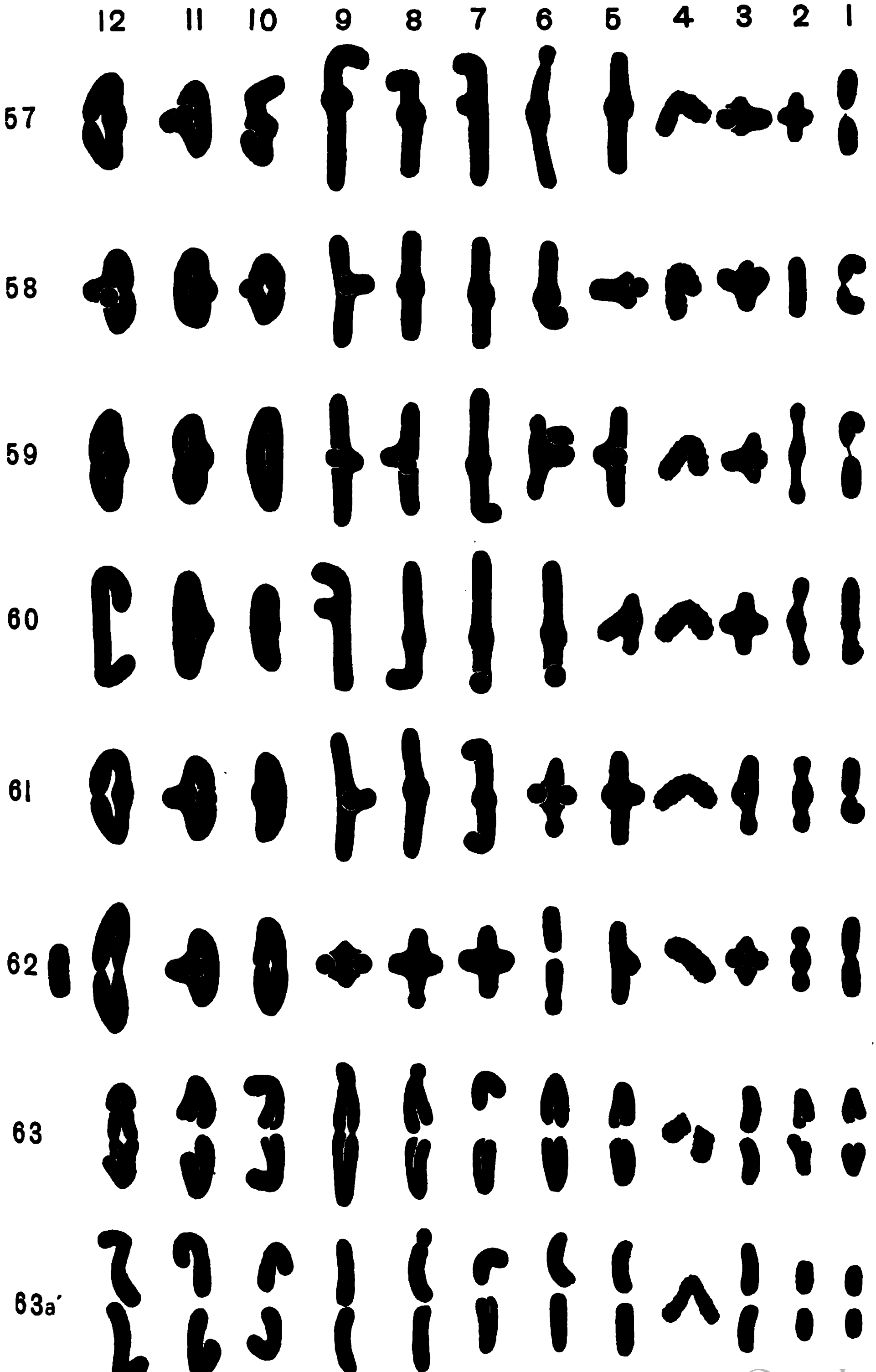

63a′

PLATE 10

EXPLANATION OF FIGURES

Side views of eight first spermatocyte metaphases of Circotettix lobatus. Note that there are only eleven chromosomes, number 2 being absent. None of these individuals have less than four rings of the Stenobothrus type.

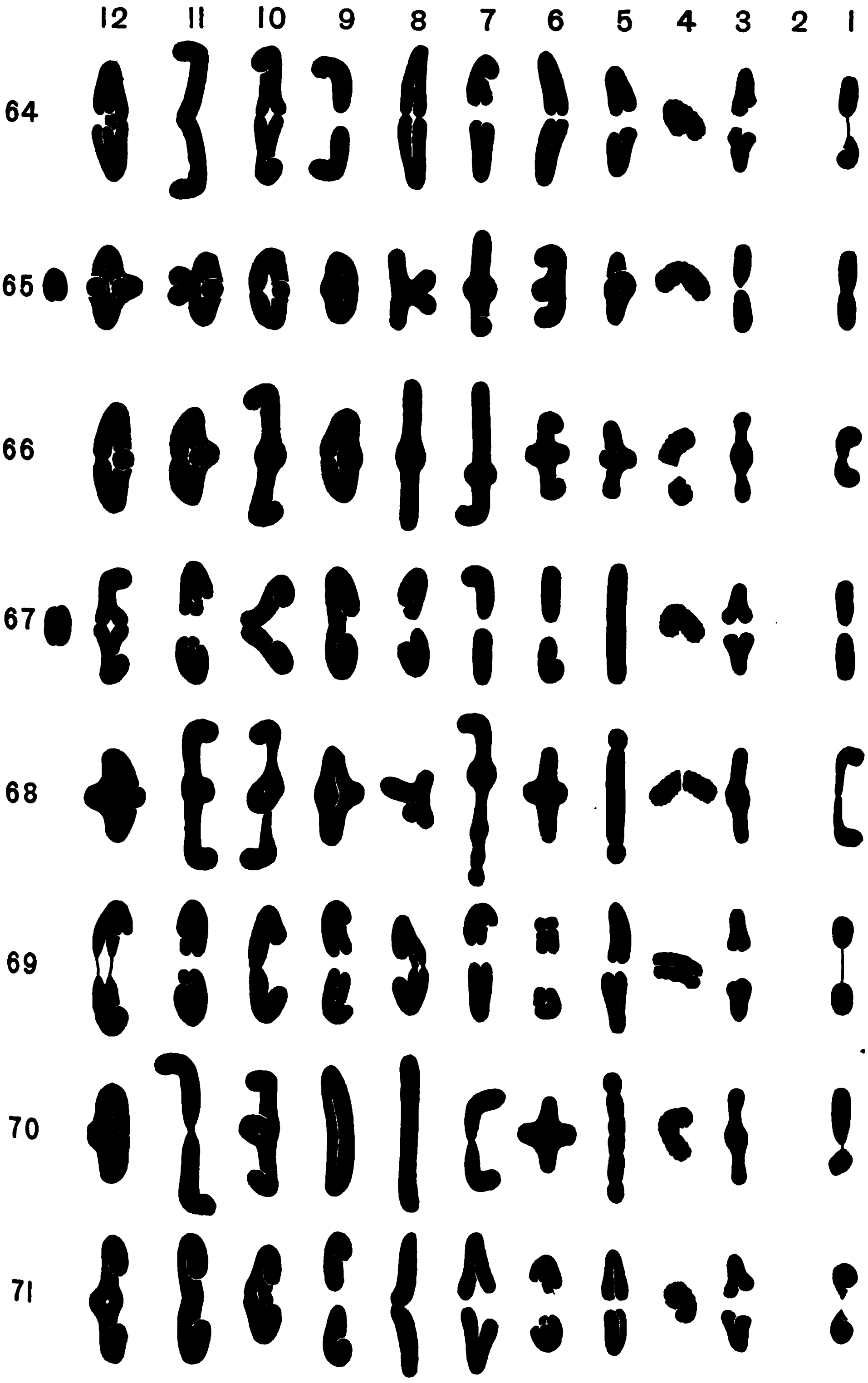


PLATE 11

EXPLANATION OF FIGURES

Spermatogonial and somatic metaphases.

1*i* From the same animal as plate 1, twelve atelomitic chromosomes.

22*a* Seventeen atelomitic chromosomes. For first spermatocyte, see plate 4, 22.

32*a* Fifteen atelomitic dyads. For rearrangement and comparison with first spermatocyte, see plate 5, 32 and 32*a*′.

33*a* Ten atelomitic chromosomes.

38*a* A tetrad dividing unequally.

62*a* Seven atelomitic chromosomes, the minimum number.

62*b* Another complex from above individual.

63*a* Eight atelomitic chromosomes. For rearrangement and comparison with first spermatocyte, see plate 9, 63 and 63*a*′.

70*a* Circotettix lobatus. Entire complex, twenty-one chromosomes.

70*d* Prophase tetrad showing vesicles on one homologue.

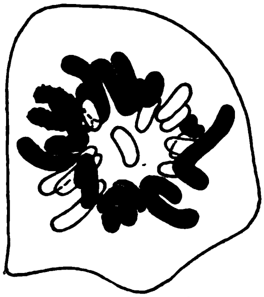
70*e* Unequal division of same tetrad in first spermatocyte metaphase.

75 Circotettix lobatus. Entire complex, twenty-one chromosomes.

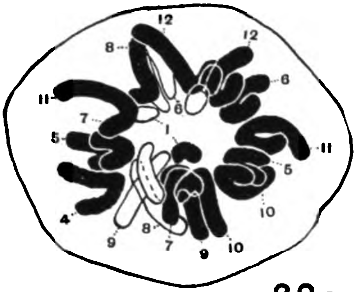
72 Somatic complex from a female of form 'B,' ten atelomitic chromosomes.

73 From another female of same group, eight atelomitic chromosomes.

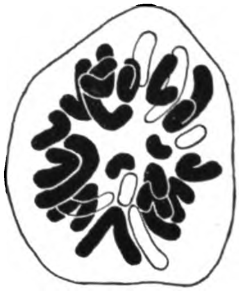
74 From a female of form 'A,' fourteen atelomitic chromosomes.



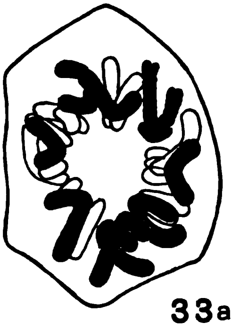
1 i



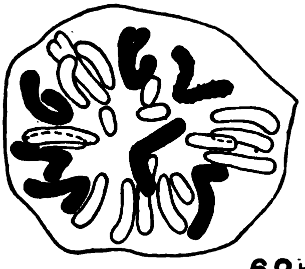
32 a



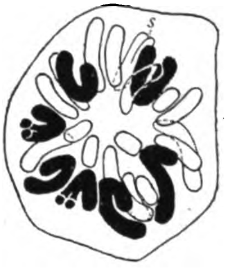
22 a



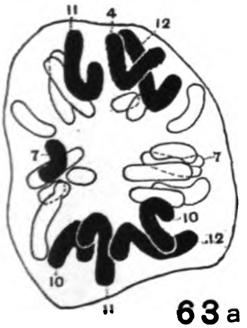
33 a



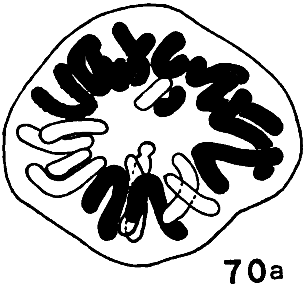
62 b



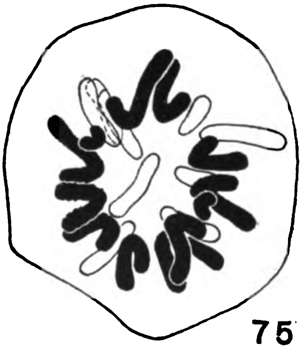
62 a



63 a



70 a



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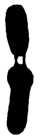
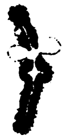
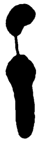


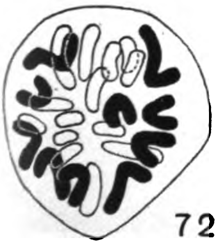
38 a



70 d



70 e



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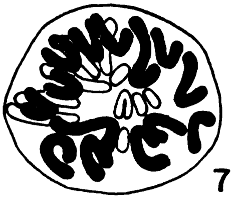

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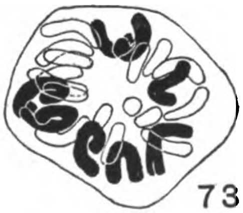


73

PLATE 12

EXPLANATION OF FIGURES

Second spermatocyte metaphases from animal number 1 showing twelve morphologically different types, as follows:

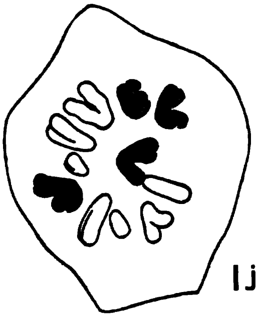
Eleven chromosomes

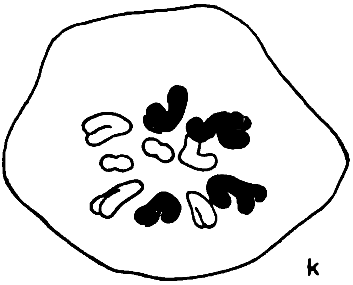
1. *j*, four V's plus seven rods.
   k, four V's plus six rods plus V of tetrad number 7 or 8.
   l, four V's plus six rods plus V of tetrad number 1.
   m, four V's plus five rods plus V's of numbers 1 and 7 or 8.
   n, four V's plus five rods plus V's of numbers 7 and 8.
   o, four V's plus four rods plus V's of numbers 7 and 8.

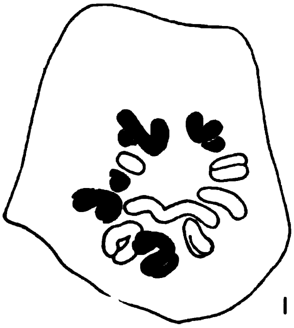
Twelve chromosomes

p, five V's plus seven rods.
q, five V's plus six rods plus V of tetrad number 7 or 8.
r, five V's plus six rods plus V of tetrad number 1.
s, five V's plus five rods plus V's of numbers 1 and 7 or 8.
t, five V's plus five rods plus V's of numbers 7 and 8.
u, five V's plus four rods plus V's of numbers 1, 7 and 8.

For first spermatocyte metaphases from this animal, see plate 1, and for spermatogonia plate 11, 1 *i*.


1j


k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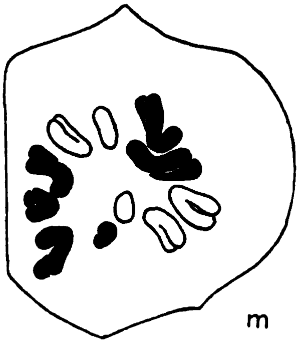
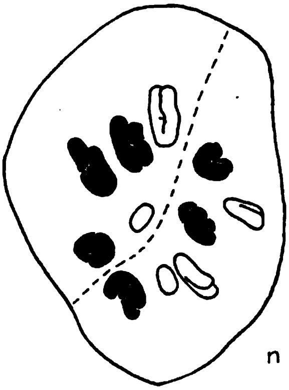

m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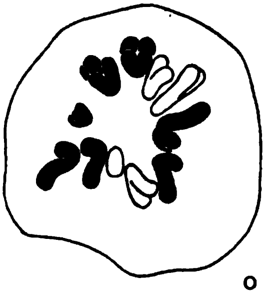
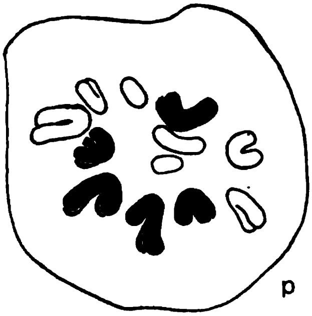
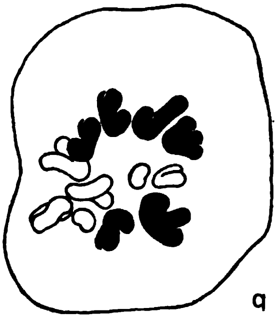
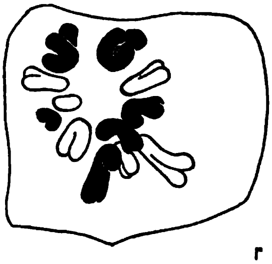

o


p


q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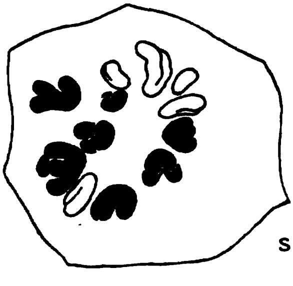
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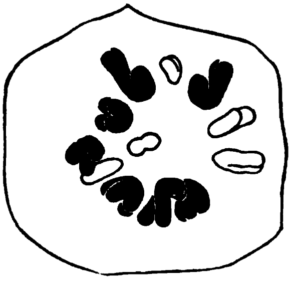
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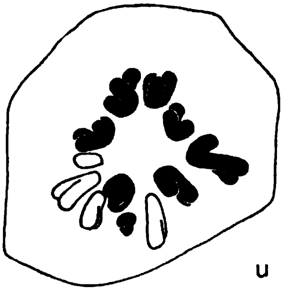

u

PLATE 13

EXPLANATION OF FIGURES

Second spermatocyte metaphases.

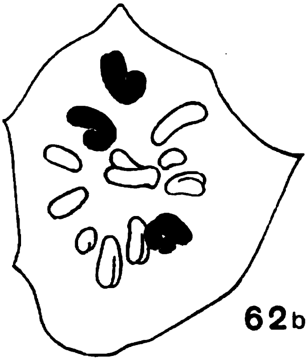
62*b* to *c* From the individual with the minimum number of atelomitic chromosomes, seven in spermatogonia (Plate 11, 62*a*). For first spermatocyte see Plate 9, 62. There are four types owing to the presence of the supernumerary as follows:

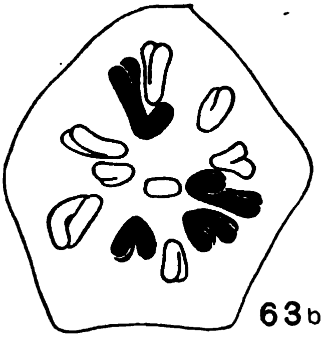
b, eleven chromosomes.
c, eleven chromosomes plus supernumerary.
d, twelve chromosomes.
e, twelve chromosomes plus supernumerary.

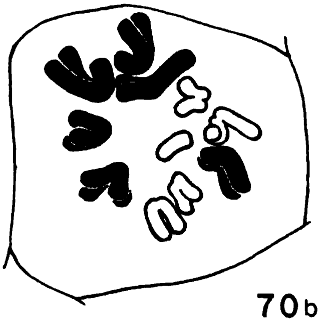
63*b* to *e* The four classes of second spermatocytes formed by individual 63.

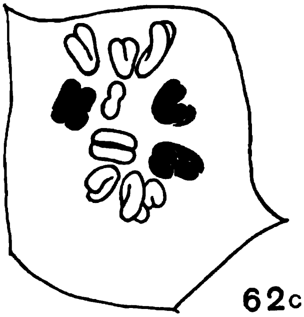
69 Circotettix lobatus, eleven chromosomes.

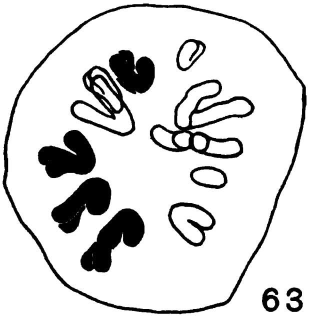
69*b* Same, ten chromosomes.


62b


63b


70b


62c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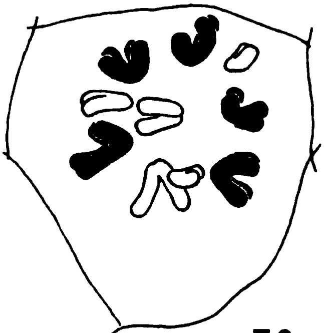
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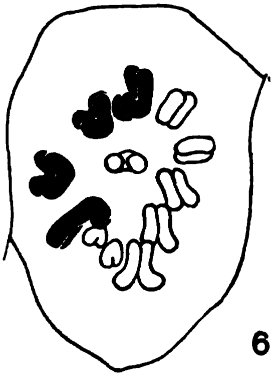
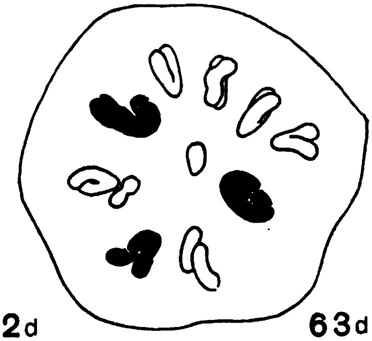
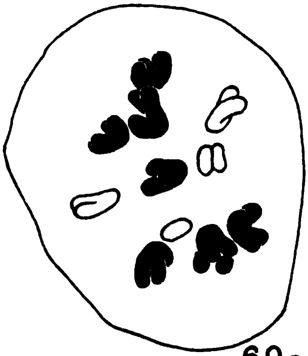
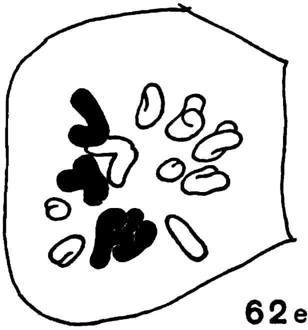
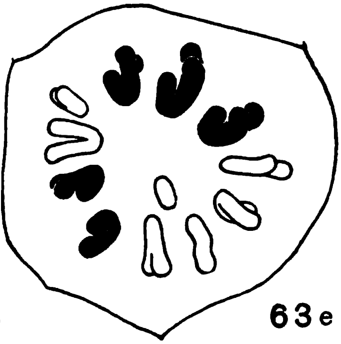

62d


63d


69a


62e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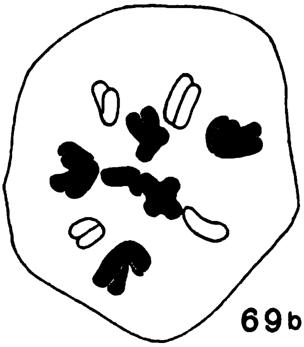

69b

PLATE 14

EXPLANATION OF FIGURES

Photomicrographs

21a Homomorphic constricted type of chromosome number 3.

62f Heteromorphic form of same chromosome from another individual—one homologue constricted the other smooth.

13a Homomorphic smooth type of same chromosome from a third animal.

1v The concurrent passage of the atelomitic homologues of chromosomes number 7 and 8 to the opposite pole from the accessory in specimen number 1.

1w The same except that the atelomitic dyads are passing to the same pole as the accessory.

1x The three J-shaped tetrads of animal number one with alternate distribution of atelomitic dyads of chromosomes number 7 and 8.

22b Homomorphic, atelomitic form of chromosome number 1.

22c Heteromorphic condition of chromosome number 11.

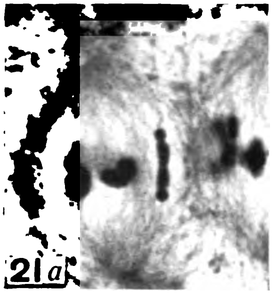
23a Homomorphic long-armed form of chromosome number 11.

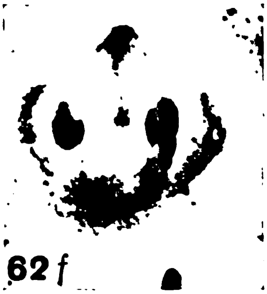
10a Homomorphic short-armed form of chromosome number 11.

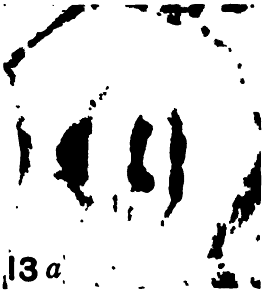
22d Heteromorphic condition of chromosome number 9.

17a Homomorphic long-armed form of chromosome number 9.

10b Homomorphic short-armed form of chromosome number 9.

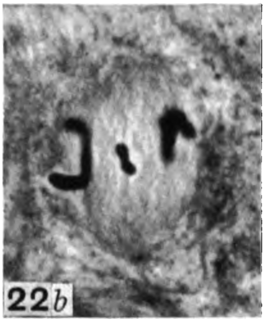

21 *a*

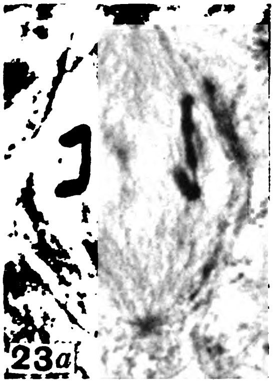

62 *f*

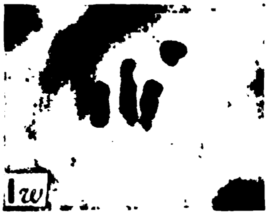

13 *a*

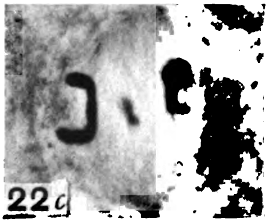
1 *x*

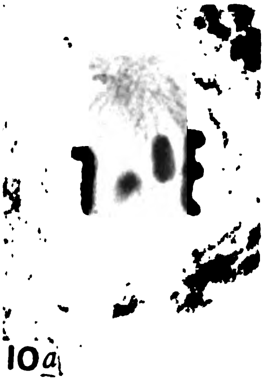
1 *v*

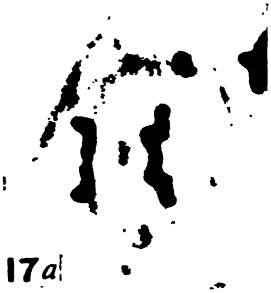

22 *b*

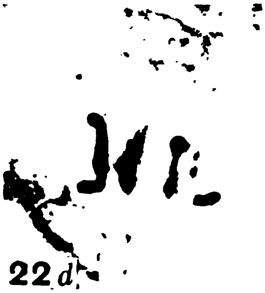

23 *a*

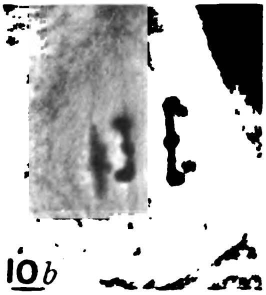

1 *w*


22 *c*


10 *a*


17 *a*


22 *d*


10 *b*

[*Reprinted from* SCHOOL AND SOCIETY, *Vol. II, No. 28, Pages 69–72, July 10, 1915*]

THE BEARING OF HIGH-SCHOOL SCIENCE ON A COLLEGE COURSE IN GENERAL ZOOLOGY

THE American Society of Zoologists, Eastern Branch, in 1903 after a lengthy discussion, appointed a committee to draw up the content of a high-school course in zoology that would lead to a one-unit college entrance credit. This was reported and approved at the Christmas meeting in 1904. Beside the outline of the proposed course a recommendation was added that students intending to take zoology in college be advised not to take zoology at school but devote their time in its place to physics and chemistry.

It has long been a subject of discussion whether a science as taught in the high school has any value as a preparation to future college work in that science. A "feeling" that one hears often expressed by science teachers of college freshmen is that they would rather have a student come to them without ever having had previous experience with the science than one who has had such a course in the high school. For this "feeling" is there any justification?

The zoological department of the University of Pennsylvania has for the past four years made a record of the previous history of its students. As the numbers seemed at this time large enough to have some significance, the record of two hundred and sixty-eight students being available, a table was made showing the distribution of grades from different schools.

It was thought that a comparison of the students from public high schools, most of which teach biology in some form when compared with private high schools, few of which teach any biological science, some conclusions might be formed. In collecting the data it was found that the Philadelphia high schools gave about one third of the students and formed rather a natural group. About a third were from other public high schools located outside of Philadelphia. The great bulk of these were high schools in the smaller cities of Pennsylvania and New Jersey. The other third were from private schools scattered throughout the east but mostly located in the neighborhood of Philadelphia. These three groups being of nearly equal size (eighty-four, ninety-six and eighty-eight students, respectively) they were readily comparable.

The grades in use at the University of Pennsylvania are as follows: D means distinguished; G, good; P, passed; N, not passed; F, failed. For our purpose F and N are combined. The decimal rating of these marks varies with the instructor, but it is generally the practise to let P be above 60 and below 75. The important point to note is that there are four arbitrary classes into which students are placed supposedly in the order of their performance.

For ease of comparison the percentage frequency is given and in some cases in the tables the two upper and the two lower marks are combined and their difference also expressed in per cents.—plus if the higher marks form the larger class or minus if the smaller marks form the larger class.

TABLE I

The Per Cent. Distribution of Grades Received in General Zoology by Students of Different Classes of Schools. Data for Four Years

Schools	No.	% D.	% G.	% P.	% N.
Phila. high schools..........	84	15.1	43.0	35.6	6.0
High schools outside of Phila.	96	1.0	23.0	57.0	18.7
Private schools............	88	1.1	13.6	46.6	38.6

Three tables have been compiled. Table I. shows the percentage frequency of the students in general zoology for four years according to the three groups mentioned above. Table II. is based on a census of the class 1914–15. This question was asked: "What sciences did you take during the last three years of high school?" The answers to this question are tabulated with respect to the mid-year grade in general zoology. The Philadelphia high schools being more of a unit than either of the other groups are considered with respect to presence and absence of zoology, botany, physics and chemistry, while in those high schools outside of Philadelphia and the private schools the presence or absence of zoology is alone presented. In Table III., the mid-year grades of freshmen in zoology, in Latin and in mathematics are compared. The

TABLE II

This table is the result of a census of this year's class in General Zoology. It shows the effect of the previous work taken by the student on his mid-year grade. Only the last three years at school are considered.

Students from		Mid Year Marks in General Zoology			
		No.	% D. and G.	% P. and N.	% Dif.
Phila. high schools	+Zoology	19	79	21	+58
" " "	−Zoology	16	36	64	−28
" " " +Chemistry	−Zoology	14	43	57	−14
+Physics	−Zoology	18	33	66	−34
" " " +Botany	−Zoology	7	57	43	+14
" " " −Chem., −Botany,	−Zoology	2		*	
Outside Phila. high schools	+Zoology	11	18	82	−64
" " " "	−Zoology	28	18	82	−64
Private schools	+Zoology	1		*	
" "	−Zoology	14	21	79	−58

* Group in which the grades fell when the number was too small to calculate per cent.

results of Tables I. and II. are presented graphically in Fig. 1 and Fig. 2.

TABLE III

Mid-year Grades of Freshmen in Latin, Mathematics and Zoology

Students from	Freshmen	Mid Year Marks			
		No.	% D. & G.	% D. & N.	% Dif.
Phila. high schools....	Latin	21	53	47	+ 6
" " "	Mathematics	24	59	41	+18
" " "	Zoology	37	60	40	+20
High schools outside Phila..............	Latin	31	35	65	−30
High schools outside Phila..............	Mathematics	32	37	63	−26
High schools outside Phila..............	Zoology	38	13	87	−74
Private schools.......	Latin	20	50	50	0
" "	Mathematics	17	35	65	−30
" "	Zoology	22	18	82	−64

In drawing conclusions from these data it is well to remember that in many cases the numbers are small and the probable error therefore very large.

The results of an inspection of these tables are as follows: (1) Table I., poor showing made by students of private schools is most apparent; (2) Table II., students from the Philadelphia high schools who have had a course in zoology make the best showing; (3) the study of botany in high school is an advantage; (4) chemistry and physics are no advantage to a student as far as grades are concerned in this course of college zoology; (5) in the high schools outside of Philadelphia in the long run it makes no difference whether they have had zoology or not; (6) in the private schools zoology is rarely taught. Because the bad showing made by private schools may be due to the fact that they do not teach zoology the mid-year grades of the same class were

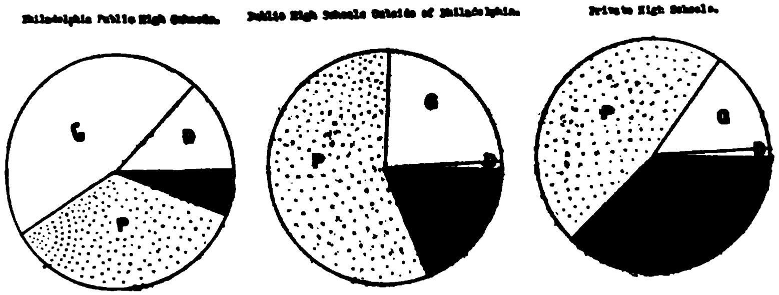


FIG. 1. Freshman zoology-marks for four years.

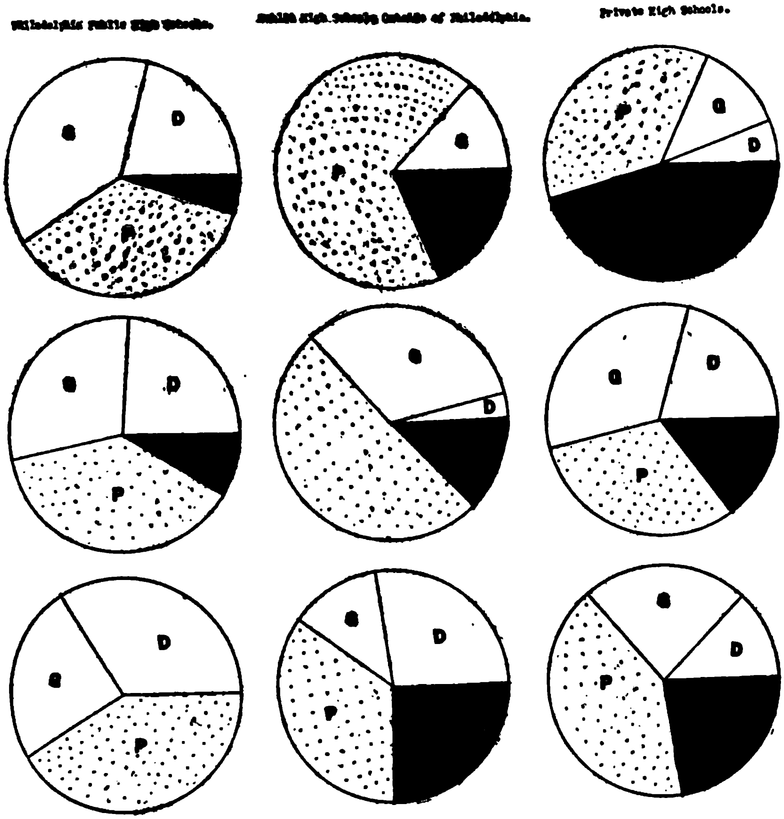


FIG. 2. Upper: mid-year marks in freshman zoology. Middle: mid-year marks in freshman Latin. Lower: mid-year marks in freshman mathematics.

investigated for two other subjects, subjects that are supposed to be well taught in private schools, viz.: Latin and mathematics. The distribution of the mathematics grades is more like that for zoology. (7) Students from the private schools, however, do as well in Latin as those from the Philadelphia high schools.

To return to the recommendation of the American Society of Zoologists made in 1904, students intending to study zoology in college were to be advised not to study zoology in school but devote their time to physics and chemistry. The supposition should be that chemistry for example should be an advantage in their college zoology course. Our figures show it does not lead to better marks.

If we recommend this ought we not arrange our course so that a preliminary knowledge of physics and chemistry would be a greater advantage to the student than a preliminary knowledge of zoology?

Indeed the author does not know if these conclusions from the University of Pennsylvania will generally hold. It would be of great interest if similar investigations could be undertaken at some of the older private universities and at some of the state universities. It is only by such an investigation that any real conclusions can be formed. We can not form opinions by recalling to our mind a few individual cases no matter how vividly they may stand out. If we make recommendations designed to apply to the "general run" of students, these must be based on an examination of the "general run."

The author does not wish to let the bad showing of the private school pass without a remark. From the public school we have entering the university a number of selected men, the survival of the fittest both in intellectual ability and in a very much less degree in the pocketbook of the parent. In the private school on the other hand there is little selection. The parent in almost every case can send his child to college. Therefore these schools have in many cases become cramming machines. In the records many students reported having been to a public high school before they entered a private school. In most cases these were very poor students, so that one can not help but infer that they were eliminated from the public school and crammed in the private. In that way the intellectual product of the private school has become much lowered.

Again another test of this side of the private school may be made by an examination of the grades in Latin. These poor students very rarely take Latin. The result then is that the private schools make as good a showing as the city public schools.

One can not fail to conclude from these figures even allowing for the probable error that it is more advantageous for a student in the general zoology course at the University of Pennsylvania to have had some biology in school and as the course is at present carried on there is no incentive for a student to come prepared in both physics and chemistry as recommended by the American Society of Zoologists. The author sincerely hopes that similar statistics can be gathered from other institutions so that we can see how generally this state of affairs exists, and, if necessary, what remedy can be worked out to eliminate it.

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[*Reprinted from* SCIENCE, *N. S., Vol. XLII., No. 1079, Pages 307–308, September 3, 1915*]

ANOTHER REASON FOR SAVING THE ~~SPECIES~~ Genus

I AM writing to second Dr. F. B. Sumner's plea for the saving of the genus.[1] I am sure he has the sympathy of the great mass of workers in non-taxonomic biology. Leaving aside the question of expressing relationship in the generic name which Sumner has so well stated, there is another point that he has not sufficiently emphasized. It is by the genera that animals and plants are catalogued. In the *Nautilus,* Vol. 28, February, 1915, the writer made this plea. I illustrated it by the form on which I had been working for the past eight years, the genus *Lymnæa.* I quote the following passage from that paper:

> The most recent classification of this group is that of F. C. Baker in his admirable "Lymnæidæ of North and Middle America" (Chicago Academy of Sciences Pub. No. 3, 1911), p. 120. Whereas the older classifications considered shell characters alone, this author "proposed to classify the Lymnæids by the characters of the shell, genitalia (shape of prostate, relative size and form of the penis and penis-sac) and radula."
>
> On a basis of these criteria he has split the genus *Lymnæa,* as defined by Haldeman, 1840, Gould, Binney, 1868; Dall, 1871; Tryon, 1872 and 1884, and more recently by Dall in 1905, into six genera: *Lymnæa, Pseudosuccinea, Radix, Bulimnæa, Acella* and *Galba.* He has done this mainly by raising a number of subgenera and sections of former authors to generic rank. I wish to ask this question: Is this at the present time justifiable? (1) Baker lists 103 species and varieties of the old *Lymnæa* in this work. Of but 33 have any-

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[1] "Some Reasons for Saving the Genus," SCIENCE, Vol. XLI., No. 1068, p. 899.

thing of the anatomy, radula and genital organs been studied. Therefore the shell characteristics are the important ones after all. (2) All these new genera are based largely on quantitative characters. The only qualitative character mentioned is the radula and this is given quite a subordinate place in the classification. (3) In his diagnosis of the genus *Galba* in his key he states that the "Penis" (epiphallus) is shorter than the "penis-sac" (penis). However, for two of the species of this genus the epiphallus is longer than the penis. (See Baker, pp. 263 and 277.)

In the mind of the writer our present knowledge will not allow us to make a comprehensive classification of the *Lymnæidæ* based on the anatomy of the snail. We know too few species well. On the other hand, the shell characters alone in a mollusk with such a generalised form of shell as have the Lymnæida are not characters on which one can base much reliance. On account of these reasons the writer would make the recommendation that the old genus *Lymnæa* should be retained in the sense that it has been used for the past seventy years.

In the *Nautilus* for June, 1915, Mr. F. C. Baker answered the writer in an article entitled "On the Classification of Lymnæids." I think this may be taken as the typical attitude of a taxonomist. He said:

> The writer can by no means agree with the statement made twice in this paper (*loc. cit.*) that generic names should not be added unless based on undebatable grounds, because of the inconvenience of the cataloguer. If this criticism should be recognized we should revert to the use of many of the older names in the Pulmonata as well as in the Naides.
>
> It is recognized, of course, that generic subdivisions can be overdone, but in the advancement of science the convenience of the cataloguer or teacher is not considered.
>
> We welcome all additions to knowledge and we know full well that the work of yesterday is rendered obsolete by the work of to-morrow, but the writer can not see how the reduction to subgenera and sections of the names used as genera and sub-

genera in the monograph in question advances our knowledge of the family any more than the raising of a number of subgenera and sections to generic rank, as Colton believes the writer to have done in his monograph. This rather resembles a game of see-saw.

This whole discussion hangs on the question, is it necessary to change generic names to advance our knowledge? The writer believes that to change generic names without an overwhelming amount of evidence in favor of the change is hindering instead of advancing science. Species and minor groups, on the other hand, can not be too much subdivided. It is an advance to describe every variation that can be distinguished. Of this work Bateson[2] says:

> They will serve science best by giving names freely and by describing everything to which their successors may possibly want to refer, and generally by subdividing their material into as many species as they can induce any responsible society or journal to publish.

In conclusion, generic names are those by which animals are catalogued, therefore should not be changed without overwhelming evidence in favor of the change. This value of the generic name has not been sufficiently emphasized.

HAROLD S. COLTON

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On Classification in General, and the Genus Lymnæa in Particular.

A Provisional Key to the Subgenera and Species of North American Lymnæids.

BY HAROLD SELLERS COLTON

From The Nautilus, Vol. 28, February, 1915.

ON CLASSIFICATION IN GENERAL AND THE GENUS LYMNAEA IN PARTICULAR.

BY HAROLD SELLERS COLTON.

The value of the generic name is the subject of this paper. To illustrate this, the genus *Lymnaea* is considered.

The most recent classification of this group is that of F. C.

Baker in his admirable "Lymnaeidae of North and Middle America" (Chicago Academy of Sciences Pub. No. 3, 1911) p. 120. Whereas the older classifications considered shell characters alone, this author "proposed to classify the Lymnæids by the characters of the shell, genitalia (shape of prostate, relative size and form of the penis and penis-sac) and radula."

On a basis of these criteria he has split the genus *Lymnæa* as defined by Haldeman 1840, Gould, Binney 1868, Dall 1871, Tryon 1872 & 1884 and more recently by Dall in 1905, into six genera:— *Lymnæa*, *Pseudosuccinea*, *Radix*, *Bulimnæa*, *Acella* and *Galba*. He has done this mainly by raising a number of subgenera and sections of former authors to generic rank. I wish to ask this question : Is this at the present time justifiable? (1) Baker lists 103 species and varieties of the old *Lymnæa* in this work. Of but 33 have anything of the anatomy, radula and genital organs been studied. Therefore the shell characteristics are the important ones after all. (2) All these new genera are based largely on quantitative characters. The only qualitative character mentioned is the radula and this is given quite a subordinate place in the classification. (3) In his diagnosis of the genus *Galba* in his key he states that the " Penis (*epiphallus*) is shorter than the penis-sac" (Penis).[1]

However, for two of the species of this genus the epiphallus is longer than the penis. See Baker p. 263 and 277.

In the mind of the writer our present knowledge will not allow us to make a comprehensive classification of the Lymnæids based on the anatomy of the snail. We know too few species well. On the other hand the shell characters alone in a mollusk with such a generalized form of shell as have the Lymnæids are not characters on which one can base much reliance.

On account of these reasons the writer would make the recommendation that the old genus *Lymnæa* should be retained in the sense that it has been used for the past seventy years. These new genera of Baker will then become sub-genera. The subgenera of Baker will be reduced to sections with one exception, viz., that of *Simpsonia*, now *Pseudogalba*, be retained as a subgenus. This form seems to the writer, on account of the character of the radula, to be sufficiently different to cause its removal from the sub-genus *Galba*. We have then the following classification :-

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[1] I am indebted to Dr. H. A. Pilsbry for calling my attention to the fact that Baker has called the *epiphallus* the penis, and the true penis he has called the penis-sac. In this paper I will adhere to the general usage and refer to the epiphallus and penis instead of penis and penis-sac.

Genus *Lymnæa* Lamarck.

Subgenus *Lymnæa* contains the *stagnalis* group.
Subgenus *Pseudosuccinea* contains the *columella* group.
Subgenus *Radix* contains *L. auricularia*.
Subgenus *Bulimnæa* contains *L. megasoma*.
Subgenus *Acella* contains *L. haldemani*.
Subgenus *Pseudogalba* (*Simpsonia*) contains the *humilis* and *obrussa*, *umbilicata* and *parva* groups.
Subgenus *Galba* contains the section *Galba*, and the *palustris*, *catascopium* and *emarginata* groups.

The classification recommended above has certain advantages over that proposed by Baker in that it indicates differences between groups of snails but does not commit one to place a shell the anatomy of whose animal is unknown in a given genus. It can be provisionally placed in a subgenus by superficial resemblance and if later changed will not affect the generic name *It is by the generic name that animals are catalogued.* This is a very important practical matter and one that cannot be too much emphasized. To change generic names without an overwhelming amount of evidence in favor of the change is hindering instead of advancing the science. Species and minor groups on the other hand cannot be too much subdivided. It is an advance to describe every variation that can be distinguished. Of this work Bateson (Problems of Genetics, p. 249) says :- "They will serve science best by giving names freely and by describing everything to which their successors may possibly want to refer and generally by subdividing their material into as many species as they can induce any responsible society or journal to publish." It must not be thought that the writer of this is trying in any way to slight the value of Baker's work. It is a work of very great value and splendidly arranged. The clear statement of what we know of the group and what we do not know is most important. It is a model which many should follow. It has been the constant companion and guide of the writer for the past two years. He does not wish this paper to be considered a criticism but rather as an appendix to that work, making it, if possible, still more valuable to students of American mollusks.

To recapitulate : generic names are those by which animals are catalogued, therefore should not be changed without overwhelming evidence in favor of the change. This value of the genera in cataloguing has not been sufficiently emphasized.

A PROVISIONAL KEY TO THE SUBGENERA AND SPECIES OF NORTH AMERICAN LYMNAEIDS.

BY HAROLD S. COLTON.

The following is a preliminary key to some of the best known species in the genus Lymnaea. It is based on a careful study of the wealth of material in Baker's Lymnaeidae of North and Middle America. To this the writer has added a little that has come under his observation during the past eight years that he has been working with this group.

It must be noted that *L. auricularia* appears in two places in the key. This is because the radula of the Philadelphia form is different from the radula of the Chicago form.

It must be remembered also, as stated in the preceding article that the radula and character of the reproductive organs are the important characters in classification as the shell is such a generalized form. This being the case it will be of value in locating but 33 species and varieties as the other 73 have been described by shell characters alone. When they have been studied the key no doubt will have to be much modified.

The writer found it impossible to sub-divide the groups in his key. The species within a group are distinguished by shell characters alone, and although one familiar with species can distinguish them without much difficulty, it is almost impossible to state the differences in a few words.

This key is intended as a supplement to that of Baker p. 125. It makes no pretense of completeness. The writer, therefore, would be glad to hear of any practical difficulties arising in its applicaton.

In the following key the ratio between the aperture length and the shell length is referred to as the "ratio."

GENUS LYMNAEA:–

Fresh-water pulmonate mollusks. Shell : normally dextral, rarely sinistral, ovately oblong to elongate. Animal: with a short, wide, rounded foot. Tentacles : flattened. Central tooth of the radula unicuspid ; laterals bi-or tri-cuspid. Male and female genital openings separate and on the right side. Mantle margins contained within the shell.

(I) The first lateral tooth of the radula has three cusps, the others two.

The epiphallus longer than the penis. Color pattern of the mantle conspicuous. Adult with a flaring lip (Subgenus Radix) *L. auricularia* p. 179

(II) The lateral teeth of the radula with two cusps.

(1). 1st cusp grooved : epiphallus less than penis. (Subgenus *Acella*) *L. haldemani.* . . . . . p. 192

(2). 1st cusp ungrooved.

(A) Axis gyrate : epiphallus ¼ of penis—(Subgenus *Lymnæa*)—*Stagnalis* Group. . . . p. 136

(B) Axis not gyrate. Epiphallus equal or less than the penis but over ½. (Subgenus *Galba*).

(a) with not evident spiral sculpture—*Galba* Group . . . . p. 200

(b) with evident spiral sculpture.

(aa) Ratio between two and three—*Palustris* Group . . . . . . p. 298

Ratio between 1½ and two.

(aaa) Ovate shell, narrow inner lip, no true umbilicus—*Catascopium* Group . . p. 377

(bbb) Bulbous shell, wide inner lip and umbilcus—*Emarginata* Group . . . p. 408

(III) Lateral teeth with three cusps.

(1) Axis gyrate.

(A) Epiphallus less than the penis ; shell succiniform Subgenus *Pseudosuccinea*—*Columella* Group . p. 162

(B) Epiphallus less than the penis.

(a) Shell thin and transparent—color pattern of mantle visible through shell—lip flaring in adult—ratio less than 1½. (Subgenus Radix) *L. auricularia* . . . . . p. 179

(b) Shell solid buliminiform—ratio greater than 1½. (Subgenus Bulimnea). *L. megasoma* . . . . . . p. 183

(2) Axis not gyrate (Subgenus Pseudogalba).

Epiphallus usually shorter than the penis.

(aa) Inner lip flattened out and excavated. *L. umbilicata* . . . . . . p. 236

(bb) Inner lip erect. *L. parva* . . . . p. 243

(b) Epiphallus usually longer than the penis.

(aa) 10 mm. long, has 5 whorls *L. humilis.* . . . . . . p. 25

(bb) 10 mm. long, has 4 whorls *L. obrussa.* . . . . . . p. 270

ON SOME VARIETIES OF THAIS LAPILLUS IN THE MOUNT DESERT REGION, A STUDY OF INDIVIDUAL ECOLOGY.

BY HAROLD S. COLTON.

INTRODUCTION.

In this study an attempt is made to correlate some of the many color varieties of *Thais* (*Nucella, Purpura*) *lapillus* with the conditions under which the animal lives. The study is based on field work carried out in the neighborhood of Mount Desert Island, Maine, in the summer of 1915. Over 12,000 snails were collected from sixty-seven localities, sorted, and the variations tabulated. These tables were then compared with the environment of the snail.

With respect to *Thais* there is a very general belief that color is correlated with the color of the environment. Cooke (1895) cites a locality in Cornwall where striped forms are found on striped rocks. Blaney (1904) reports yellow forms were found to predominate on the yellow granite rocks of Yellow Island, a small island in Frenchmens Bay on the Maine coast. On account of the great variation of *Thais* and the apparent predominance of one variety in a given locality, a study of the variations might throw light on some of the ecological factors of the sea beach.

LIFE HISTORY OF THAIS.

Thais (subgenus *Nucella* or *Purpura*) *lapillus* is a carnivorous prosobranch mollusk. It feeds almost exclusively in this region on the mussel, *Mytilus edulis,* and the barnacle, *Balanus balanoides.* The former it attacks by boring a hole in the shell about 1 mm. in diameter, devouring the soft parts by means of its long flexible proboscis. The barnacle is eaten in much the same manner, the hole being drilled through one of the scutes. Not only do they sometimes attack the periwinkle, *Littorina littorea,* but will try to bore through the shell of one of their own kind. Although many shells show scars, there is no evidence, however, that many are destroyed in this way.

The descriptive ecologist has neglected the Atlantic coast north of Cape Cod. King and Russell's classification for the coast of Scotland will not hold for this region. Some day, if some one else has not undertaken it, the writer hopes to make a descriptive classification of the animal communities of the sea beach in the Mount Desert region. Provisionally he recognizes the following classification of the Littoral Formation:

- Littoral Formation
  - Solid Rock Association
    - Drift Zone.
    - Littorina rudis Zone (Mean High Water).
    - Balanus Zone.
    - (Fucus Zone.)
    - Mytilus Zone.
    - Transition Zone (Mean Low Water).
  - Rock Association.
  - Rock Pool Association.
  - Boulder Association.
  - Shingle or Pebble Association.
  - Sand Beach Association.
  - Mud Flat Association.

The mean rise and fall of the tide is ten vertical feet.

Thais is found in the Solid Rock, Boulder and Rock Pool Associations. In the two former it is confined to the Balanus, Fucus, and Mytilus Zones, and particularly to the Balanus and Mytilus Zones, as there the food is found. There, *Thais* is associated with *Littorina rudis*, *L. palliata*, *L. littorea*, *Balanus balanoides* and *Mytilus edulis*. When the tide is high the cunner (*Tautogolabrus adspersus*) and the pollack (*Pollachius virens*) invade this formation to feed.

Thais is not found in the Shingle, Sand or Mud Flat Associations. Nor is it found alive in any association of the Bottom Formation, unless we should classify the Rock Pool as a detached part of this formation.

It is not found in any stratum other than the upper surface of rocks. It is found therefore neither on the weeds nor under the soil.

Its habitat is very closely associated with its food. (See diagram, fig. 1, illustrating the food relations of *Thais* to its organic environment.)

The eggs are laid in little urn-shaped capsules in sheltered places. They are more abundant in the Mytilus Zone in favored spots, such as in cracks in the rocks or under masses of *Fucus* than in the Balanus Zone. In each capsule about 400 eggs are laid. As cannibalism is practised (Beard 1899, Carpenter 1857, Glaser 1905, Pelseneer 1904, Robert 1902) by the growing snail, a capsule containing snails ready to hatch will hold but 8–14.

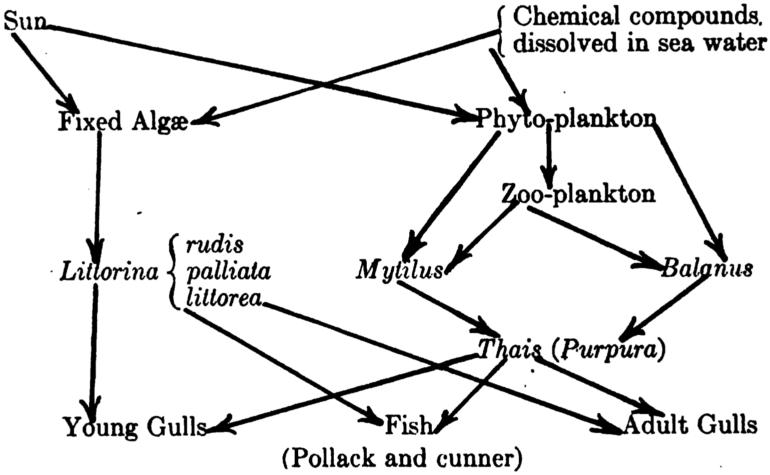

Fig. 1.—Diagram illustrating the principal relations of *Thais* to its organic environment in the Rock Association. To be complete this diagram should show an entire cycle of the material. Each one of the organisms listed above gives off liquids to the sea water that may be again used. They also furnish solids either as waste products or by death, which, when acted on by bacteria, will give more soluble matter as well as a residual solid that will help contribute to the sea bottom in other localities. The dead animals will supply food for amphipods, the amphipods food for fishes like the sculpin, and so relations are established with other associations. This diagram shows that the biota of the Rock Association is dependent alone on the palagic formation. The relationships with other associations are destructive to it rather than constructive.

The young snail has enemies in the shape of fish, so those which fail to take refuge in small cracks in the rocks are destroyed. At this time the food consists of young mussels which grow abundantly in the joints in the rocks. If one does not know where to look for the young snails, none will be seen, so far are they crowded into the cracks.

A few pollack that were caught at high water over a Thais habitat had their stomachs full of young *Littorina palliata* which they had eaten from the same locality in which *Thais* lives. *L. palliata* cannot hide in the rock cracks, as their food is on the Fucus stems. If the fish could find young *Thais*, there is no reason why they should not be eaten as well as *Littorina*.

Although the young snails are hatching throughout the greater part of the summer, most of them hatch out in August and reach between 10–15 mm. in the first winter. This is measured by large ridges on the shell, which indicate periods of rest in the growth of the shell. These rest periods are assumed to indicate the winter, but are not proved to correspond to it.

The curves in fig. 2 show the rate of growth in three different environments. These are but approximations. Further study may show that the first year may be really the second.

The oldest snail found was estimated as being seven years old. The great majority are about two or three years old.

Cooke (1895) estimates that each female snail lays two hundred egg capsules a year. Each capsule contains about four hundred eggs. Food seems to be in most places abundant. The snails have been observed to be limited in number by the following factors:

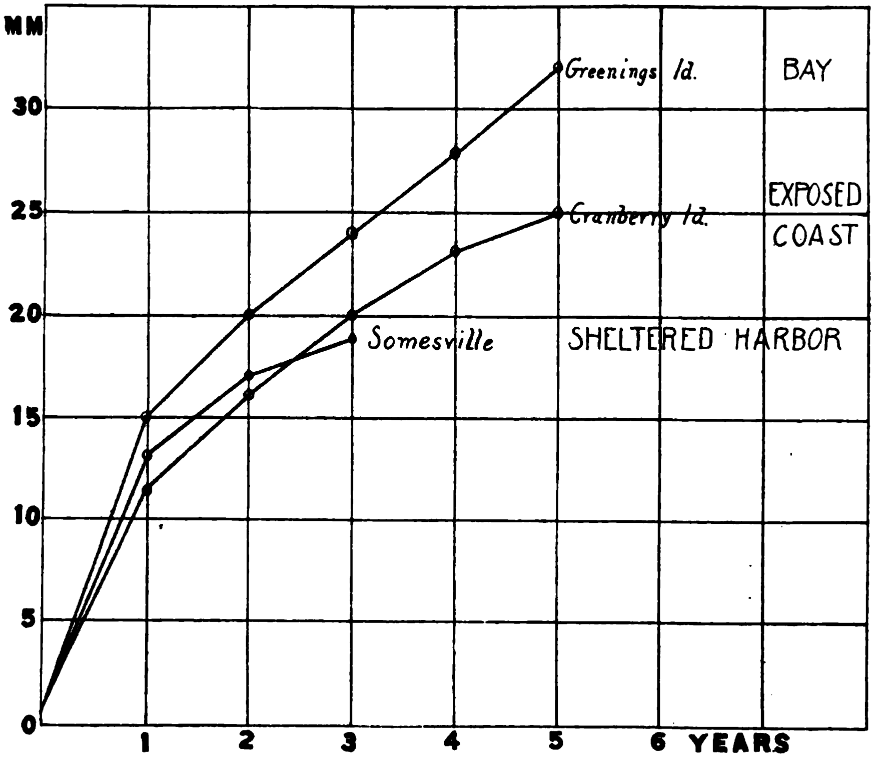

Fig. 2.

(1) Cannibalism within the egg capsule, only ten to twelve hatching out of 300–400 eggs.

(2) Limit to the number that can take refuge in cracks in rocks and yet get enough to eat.

(3) Fish, such as pollack, will gobble up any stragglers.

(4) Half-grown snails, ones too big for the pollack, are used by herring gulls as food, the empty shells being regurgitated.

(5) Very large specimens are carried by the gulls to a height and dropped on any near-by flat rock in the same way as the gulls treat both sea urchins (*Strongylocentrotus*) and welks (*Buccinum*). Only the largest *Purpura* will break and only the largest are collected for this purpose.

(6) Certain ones seem to be parasitized by a sporocyst, but this was not investigated.

(7) Accidents, such as a surf heavy enough to move the rocks on which the animals live, may destroy others. In such habitats few if any are found. The maximum size of these rocks may in exposed places be boulders two to three feet in diameter, while in very sheltered places *Thais* will be found on pebbles two inches in diameter. The limit is obviously determined by wave action.

(8) Ice probably destroys others, as they are not found in places where shore ice can accumulate.

(a) In Maine they are not abundant in shallow coves that freeze up solid in the winter time.

(b) Their geographical distribution also seems to indicate that sand beaches are a barrier, as *Thais lapillus* is not found west of Montauk Point. On Long Island Sound the barrier has not been determined. Both *Balanus* and *Mytilus* being found west of Stamford, Conn., the most westerly recorded station for *Thais*, and the shore is still rocky. In the north shore ice seemed to form the barrier See map, fig. 3.

Various adaptations are at once manifest on examining this animal: (1) The strong shell, so strong that a drop from a height will not always break it. (2) The strong foot, which makes dislodgment from its substratum difficult. (3) Its habit of seeking shelter under rocks and fucus. (4) The method of egg-laying, with its protection of the young until they are able to care for themselves, although if but two and a quarter per cent. of these laid hatch, those two and a quarter per cent. are almost sure to hatch.

METHOD.

As each station was visited, from 200 to 500 shells were collected when *Thais* was abundant. In some places they literally covered every inch of the rock. In other places they were so rare that a careful search of a quarter of a mile of beach would often disclose but two or three.

Each collection as made was placed in a bag made of mosquito netting. While still alive the snails were sorted into the dozen or so groups of the principal varieties. The number in each group was tabulated. For future reference the bag of snails was preserved in a tub of formalin.

A study of the color varieties seems to show that there are a number of colors and patterns that may be combined together in any sort of way. These are as follows:

(1) Pure white shells that show no trace of color.

(2) Purple. The color in this case is diffuse through the shell, but is darker within the aperture.

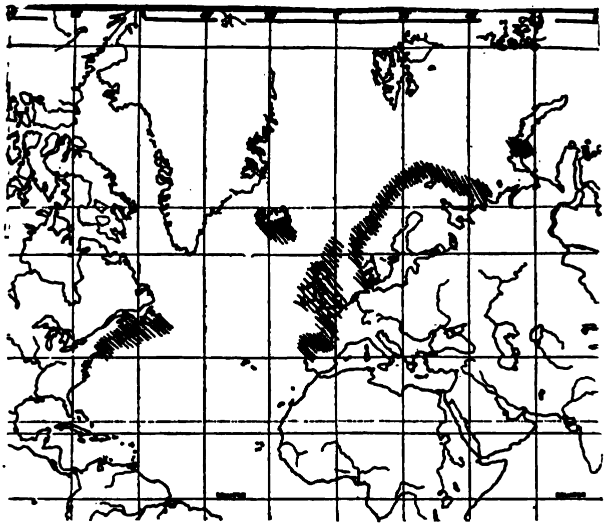

Fig. 3.—Map of the North Atlantic Ocean showing the distribution of *Thais* (*Purpura* or *Nucella*) *lapillus*. (After Cooke, Proc. Mal. Soc. London, vol. II, p. 193.) The cross-hatching shows the occurrence of *T. lapillus*. The southern barrier to *T. lapillus* is not clear, but the northern barrier is obviously the presence of ice on the shore.

(3) Chestnut. This color may be pure quite often, but it is usually combined with purple, forming a chocolate, or with yellow, forming olive.

(4) Yellow is a color which, when uncombined with one of the others, makes the shell very conspicuous on the rocks. Owing to its relative rarity and to its being so conspicuous, it often appears in shell collections in proportions that are misleading. Only by collecting all the snails from a given locality can this be avoided.

(5) *Dilute*. Every one of the colors mentioned above may appear in a dilute in contrast to its intense form.

(6) *White lip* of the apperture is quite common in some localities. In fact, purple is rarely found not associated with a trace of lighter color on the lip. This *white lip* may be combined with all colors and dilutions.

(7) *Narrow stripes.* In many shells about ten narrow white stripes are found on any color background. These are sometimes associated with the ridges which appear on many shells.

(8) *Wide stripes* of white, usually two in number, often appear. The widest stripe equals two or three of the narrow ones and the smaller stripe is double the width of the narrow ones. The widest stripe is always posterior to the narrower stripe.

(9) *Spot of purple* on the columella. This is rare at Mount Desert, but very common at Rye Beach, New Hampshire.

(10) *Imbricata.* A fluting of the ridges of the shell gives rise to the variety called by Lamarck *imbricata.*

Apparently each one of the ten characters recognized may be combined with any other. If this is so there must be 1,103 varieties. Not that many varieties have been found. This is due to the fact that one cannot distinguish certain combinations from one another. As an example, let us consider the case of the colors alone. We have purple, chestnut, yellow, and white. There are eight possible combinations of these: white, purple, chestnut, yellow, purple-chestnut, purple-yellow, chestnut-yellow and purple-chestnut-yellow. These last four are shades of olive and chocolate, and cannot be distinguished easily from one another. If we combine these four color combinations into one group and omit the white lip and purple spot on the columella, we will reduce the possible number of variations to be recognized from 1,103 to 33. Twenty-eight of these thirty-three varieties have been recognized. However, there are five that are so rare that they were not discovered in a collection of six thousand shells when they were particularly sought after. These were narrow stripe dilute yellow, wide stripe narrow stripe yellow, wide stripe narrow stripe dilute yellow, narrow stripe dilute olive, and wide stripe dilute olive. The yellows which contribute to the olive color and the dilute are much rarer than any of the others, so it is no wonder that they are the ones that would be lacking in combinations.

It is obviously impossible to attempt to correlate all the known varieties with environmental factors, so the writer has for the sake of convenience reduced still further the number of groups. The particular groups recognized are as follows:

(1) White; that is, pure white.

(2) Light—pure white, together with intense and dilute chestnut with white lip. The outer layer of the shell of these is white.

(3) Dark—chestnut and purple in all their combinations.

(4) Yellow—pure yellow.

(5) Striped—both narrow and wide stripe in all their combinations and color.

(6) Imbricated—in all the combinations of patterns and colors.

In the tables that follow the number of snails found in each group is stated in per cent. of all the snails in each collection from a given station.

THE RELATION OF THE VARIETIES TO THE ENVIRONMENT.

The base for this study was Greenings Island, an island located between Northeast and Southwest Harbors in the mouth of Somes Sound. It is triangular in shape, with a perimeter of about two and a quarter miles. The main mass of the island is composed of till, which at one time formed the terminal moraine of the continental glacier before it retired from Somes Sound. This till is protected from rapid wave erosion by outcrops of reddish granite at the east and west end of the island. This granite is cut in several places by broad basalt dykes which run in a general north and south direction. Under wave erosion the till between the outcrops of solid rocks has given material to form a boulder-strewn beach and in favorable places the finer products have given rise to pebble and sandy beaches. On the west or more sheltered side of the island between the granite outcrops are a number of muddy coves.

On this small island we have all the principal Littoral associations present. The distribution of these are found in fig. 4. This figure is a diagram of the shore of Greenings Island, with the vertical scale very much exaggerated. It has been made as though the shore were cut at the northwestern point and folded out so that this northwestern point appears at each end of the diagram. The character of the shore is indicated. The island as a whole is protected from heavy surf by outlying islands, but the eastern end is exposed to the ocean swell from both the south and east, and at times of storms is subject to quite a surf. The northeast shore and south shore are relatively unprotected at the east end, but become more protected toward the west. On the diagram the per cent. of pure white shells are placed in the locality in which the collection was made.

An inspection of this figure will show that the most unprotected portion has the lighest colored snails, while the portion of the island

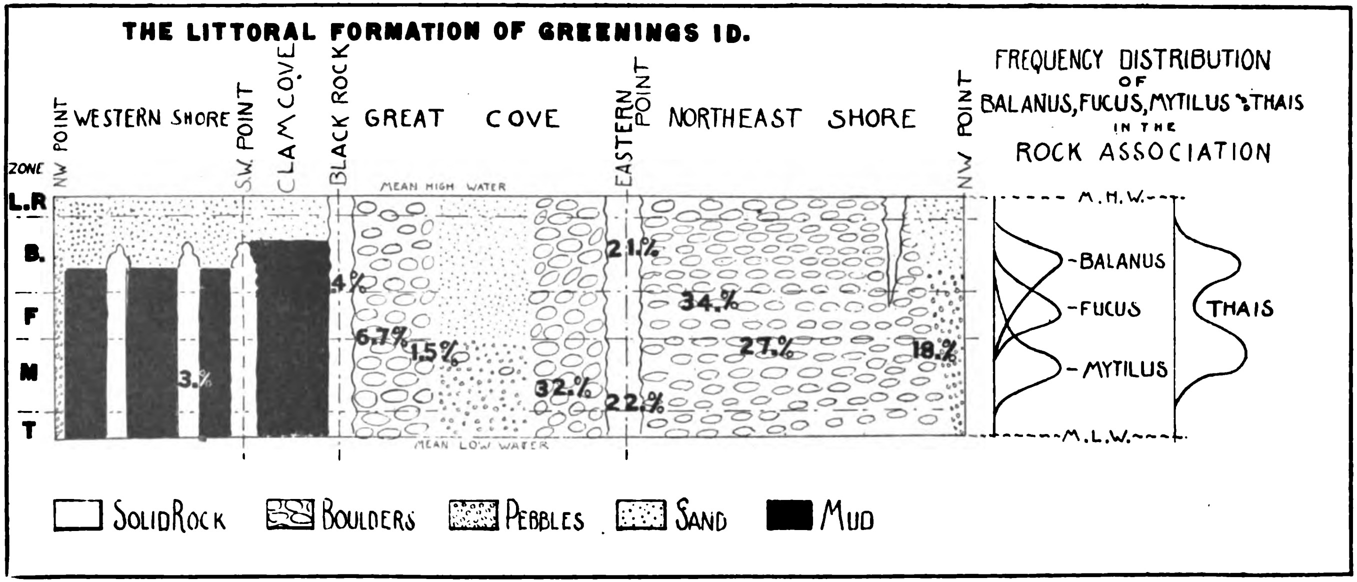

Fig. 4.—Diagram of the beach of Greenings Island, Maine. This is drawn as if the shore of the island were cut at the northwestern point and beach folded out into a straight line. The length of the diagram represents two and a quarter miles. The width represents the space between mean low tide and mean high tide, which is equal at this place to ten vertical feet.

The percentages on the diagram refer to the per cent. of white *Thais* found at each locality. It shows how the number of white are most abundant at the eastern end and fewest at the western.

L. R., Littorina rudis zone; B., Balanus zone; F., Fucus zone; M., Mytilus zone; T., Transition zone.

subject to the smoothest water has the darkest. This difference is very striking and the intermediate stations show intermediate ratios.

This has led the writer to classify the shores according to the waves. These are arbitrary divisions and of course every intergradation is found, but in most cases there is very little hesitation how a given station should be described. If a great uncertainty exists, the station was placed in both classes.

Sea cliff or surf exposed to the open sea. An ordinary row boat can practically never land with safety.

Bay exposed to a moderate surf during high winds. Except at those times a small boat can easily land on the shore without danger of accident.

Harbor so smooth that a row boat can practically always make a landing.

In the tables which follow the stations nearest together are always compared.

TABLE I.

Locality.	Condition.	Station No.	No. of Individuals.	% Lt.	% White.
Bakers Id	Surf.	23	65	6.1	0
	Bay.	24	167	20.7	6
Seal Harbor	Surf.	54	176	2.8	0
	Bay.	56	34	55.6	44
Cranberry Id	Surf.	10	319	30	4.3
	Surf.	11	195	59	9.7
	Bay.	13	204	15	5.7

We can conclude that in general the snails on the most exposed situations are darker than those found in the bay.

TABLE II.

Locality.	Condition.	Station No.	No. of Individuals.	% Lt.	% White.
Greenings Id	Bay.	2	516	44½	21½
	Harbor.	31	333	10	1½
Buckel Id	Bay.	15	234	63	36
	Harbor.	16	218	13½	5½
Bear Id	Bay.	43	277	24	12½
	Harbor.	44	67	10½	1½
Orono Id	Bay.	19	55	11	7
	Harbor.	18	184	50	27
Southwest Harbor	Bay.	37	194	29½	19
	Harbor.	36	196	10½	5½
	Harbor.	35	273	25½	11
	Harbor.	47	172	14½	6½

If we combine the conclusion from Table I with Table II we see that snails in the exposed positions tend to be dark as well as those in the sheltered places, while those in the bay shore tend to be the lighter colored.

The following table includes the results of collections made at stations separated by no barrier, near together, but on a substratum of different colored rocks. The numerous black basalt dykes that everywhere cut the red granite make a number of collections of this sort possible. Only a very few of the possible ones have been studied.

TABLE III.

Locality.	Condition.	No. of Station.	No. of Indiv.	% Lt.	% White.	% Yellow.
Greenings Id., east end.	Red rocks, 1000 ft. to Sta. 2.	25	316	57	34	6.3
	Black rocks, 20 ft. to Sta. 50.	2	516	44	21	0
	Black rocks, 500 ft. to Sta. 21.	50	160	37	22	0
	Red rocks,	21	164	47	32	.6
Greenings Id., southwest end.	Red rocks, 200 ft. to Sta. 28.	31	333	10	1.5	2.1
	Black rocks, 50 ft. to Sta. 30.	28	247	14	6.7	1.2
	Black rocks,	30	289	2.9	.4	3.8
Suttons Id.	Red rocks, 110 ft. to Sta. 8.	9	159	35	2.5	7.5
	Red rocks, 150 ft. to Sta. 7.	8	135	28	2.2	31.8
	Black rocks.	7	123	19	0	18.7

In general, more light-colored snails are found on the red rocks than on the black rocks. The association of yellow forms with the environment is inconclusive. There does not seem to be any correlation from these few cases. Thirty-three collections from red rocks average 4% yellow. Fifteen collections from black rocks average 4.7% yellow.

In this region there are occasional outcrops of a banded rock, a chlorite schist. That there might be a correlation between striped forms and schist is a possibility that may be investigated. The collections from the red granite given in the table are those made nearest to the chlorite schist. This comparison was not in mind when the collections were made, so the stations are often several miles apart.

TABLE IV.

Locality.	Substratum.	Striped Shells.
Blue Hill Bay	Chlorite schist	1.1%
	Red granite	9.8%
Western Way	Chlorite schist	27 %
	Red granite[1] (average of 18 collections from this region)	14.4%
Western Way	Chlorite schist	27 %
Nearest Granite Station	Red granite	18.5%

The distance apart of the stations introduces so many other factors that with such meagre data no conclusions can be drawn. The writer expects that the results are really negative.

The relative proportions of the imbricated forms from different localities are as follows:

In the upper bays the water is much warmer than near the ocean. This warmth is indicated at Blue Hill by bathhouses being located along the beaches. None of the resorts farther down the bays have any, the water being too cold for bathing in the bay in those places. The upper bays are relatively shallow and there are muddy spots found on the rocky beaches between the rocks. Five stations in this region had imbricata ranging from 38% to 96%.

In the middle bay region of Blue Hill Bay the water is deep and the rocky shores are clean. No imbricata were found in the three stations in this region.

The western side of lower Blue Hill Bay is shallow, with many islands and ledges. Among these islands there are many mud flats, and the same conditions are found on the upper bay. Five stations had *imbricata* ranging from 9% to 30%.

Near the islands separating the lower bay from the ocean the water is, in general, deep and the rocky shores clean. Five stations, 0–5½% *imbricata*.

On the outer islands the surf, of necessity, keeps the rocks free from sediment. Two stations, 17–57%.

In Somes Sound environmental conditions similar to those in Blue Hill Bay are found. The rocks are muddy in the harbors near mud flats and clean where wave action is possible and the water is deep. If we except two stations at the Southwest Harbor steamboat wharf, where the ratio of *imbricata* was 19% and 19.2%, respectively, the average of thirty-five stations containing 7,119 individuals was only .47%. The largest number found was but 3½% at any one station and that was on the rocks of a muddy harbor. The station in Somes Harbor farthest away from the ocean had but .6% *imbricata*.

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[1] Of these eighteen collections, four had over 27% striped, one had 37% striped.

That there are other geographical factors is shown by the rarity of *imbricata* in the Somes Sound region. Very few are found in exposed situations on the Cranberry Islands and on Seal Harbor Head, situations exposed to the full effort of the surf. Except for a colony at the Southwest Harbor steam-boat wharf, in the whole Somes Sound region they are indeed rare. Out of 2,765 shells collected on Greenings Island but three were imbricated and these three were found at one station on the muddy west side of the island.

However, in general, we can say that the imbricated forms are found in greatest abundance in exposed situations, as Duck Island and Long Island, and also in the most sheltered positions.

Most of the varieties found in the Atlantic are also found in the forms inhabiting the north Pacific Ocean. Dall[2] reports that the lamellated forms of *Thais* in that region are found best developed in sheltered harbors. The Atlantic lamellated forms we have seen may occur in sheltered as well as in exposed situations.

As *Thais* is found in both the Balanus and the Mytilus Zones, is there any difference between collections made close together but in different zones? The answer to this question is summed up in the following table (Table V) compiled from collections made but a few feet apart, but in different zones.

TABLE V.

Locality.	Zone.	Sta.	Lt.	Wt.	Yel.	St.	Imbricata.
Greenings Id., eastern point	*Balanus.*	2	44.5	21.3	0	?	0
	Mytilus.	50	37.5	22.5	0	14.4	0
Steam-boat wharf, S. W. H.	*Balanus.*	37	29.5	19.	17.5	1.5	19.0
	Mytilus.	47	14.5	6.4	4.1	37	19.2
Stanley House wharf, S. W. H.	*Balanus.*	35	25.3	11	2.2	4.0	0
	Mytilus.	34	21.4	4.8	0	5.3	0

(1) There are more light forms in the white Balanus Zone than in the dark Mytilus Zone.

(2) There are fewer yellow forms in the dark Mytilus Zone.

(3) Striped forms not conclusive.

(4) Imbricated forms not affected.

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[2] *Proc. U. S. Nat. Mus.*, vol. 49, pp. 557–572.

NATURAL SELECTION.

Natural selection, we can assume, is most severe on Little Duck Island. This little island, lying four miles out at sea, is exposed to the surf on every side. The herring gull, one of the chief enemies of adult *Thais*, has a rookery on the island, and every summer thousands of birds roost there at night and have their nests. Everywhere regurgitated Thais shells cover the rocks, which tell a story of a struggle for existence.

In collecting on this island for two hours but four hundred and twenty-nine living snails were found. These were discovered at the lower edge of the Mytilus Zone. All had been picked off the rocks above by the gulls. This collection is chiefly notable in containing a very large proportion of dark individuals, only 1.1 per cent. white were found. Nineteen per cent. were imbricated.

It can be said with justification that where the struggle for existence is greatest on the exposed islands and the muddy harbors selection tends to preserve the dark-colored and the lamellated forms.

CONCLUSIONS.

A study of the color varieties leads one to the conclusion that they are hereditary and that we are dealing with things that are probably unit characters. Even the development of lamellæ, as in *imbricata*, are not modifications caused by induction that is caused by the direct action of the environment.

As there are great differences between the ratio of the color varieties present in different localities, we assume that natural selection is acting.

In the bay environment conditions are at an optimum. No mud film covers the Mytilus, and Balanus forms crusts over the rocks an inch to an inch and a half thick. Here *Thais* is most abundant. Five hundred can be gathered in ten minutes or even less. A handful can be gathered at one scoop.

In the surf environment the barnacles do not form such thick crusts. On the harbor shore ice destroys the barnacles in the winter and a mud film covers the mussels. So that the barnacles are usually small and far apart, while the mussels are separated by mud from the rocks. Sand and mud are barriers to *Thais*. Although the surf environment and the harbor environment are the antithesis of one another, yet they have many effects on *Thais* that are similar. These effects are as follows:

(1) The curve of growth, fig. 2, shows that those in the surf environment at a given age are smaller than those from the bay environment.

(2) There is a tendency toward dark individuals.

(3) There is a tendency toward lamellated forms.

These studies show that natural selection has some determining action on the color varieties present. (1) It has been shown that the color of the rocks, whether dark or light, has an effect. (2) It has been shown that the white barnacle-covered rocks contain more light-colored ones than those on the mussel bed but a few feet away. (3) It has been pointed out that those living on rocks near mud flats are darker than on the more exposed points of the same islands.

The present study must leave the question of the relation of the yellow forms and the striped forms to the color of their substratum yet unsettled.

In general, however, there is a relation between the color and the environment. This relation is caused by natural selection and not by the direct action of the environment.

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IS THE HOUSE OF TCUHU THE MINOAN LABYRINTH

WHILE going through a back number of the *American Anthropologist*[1] the writer's attention was attracted by the figure illustrated in Fig. 1. This was in a short paper by Dr. J. Walter Fewkes entitled "A Fictitious Ruin in the Gila Valley, Arizona." In this he showed that this symbol which was first observed by an eighteenth-century Spaniard scratched in the sand by a Pima Indian did not represent the plan of a ruin as previously interpreted, but was used in some way in a game "the house of Tcuhu" (Tcuhiki).

It was curious but this diagram was familiar to the writer and his familiarity came from a distant part of the world. As shown in Fig. 2 this diagram appears on the reverse of a silver coin of Cnossus in Crete of the Greek Period (B.C. 200–67). In this case the figure represents the Minoan Labyrinth. On other coins from Cnossus it sometimes appears in a square form, but even then it has the same ramifications. A comparison of this Greek coin, with House of Tchuhu when inverted, shows that the two are identical in every respect.

There are three possible explanations for the coincidence. First, these symbols may have arisen independently in the new and the old world. Secondly, the symbol may have originated in the old world and have been transported to the new in pre-Columbian times. Thirdly, that the symbol was introduced into America with the Spanish conquest.

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[1] N. S., Vol. IX., 1907, p. 501.

On the one hand, it has been pointed out by Fewkes (*loc. cit.*) that the symbol or something like it was early known to the Pima Indians, as the diagram in slightly modified form appears scratched on the adobe wall of the Casa Grande ruin among obviously Indian pictographs. On the other hand, it is possible that this diagram may have had a Spanish origin.

While it is quite generally accepted by American ethnologists that such simple forms as the cross, the swastika, the wall of Troy, etc., arose in the new world as well as in the old; yet it is hard to believe that such complicated labyrinths similar in every detail could have had separate origins. Similar environments often call forth similar responses in different organisms. In such cases the similarities when carefully analyzed are found to be superficial. The details will not agree. In this case, however, the agreement is exact.

Again there is a possible question that the figure from Fewkes is not of genuine Pima origin. A brief history of this symbol will make this clear. It seems that an unknown Spanish traveler visited the Pima country in the year 1761 or 1762. An account of this visit exists in the form of a manuscript.[2] On the margin of one of the pages of this manuscript appears the figure which I have reprinted from Fewkes (Fig. 1). According to the unknown Spaniard the Pimas draw the symbol on the sand. He stated that it represents "a house of amusement rather than that of a magnate." As no ruin has ever been discovered with such a ground plan, Dr. Fewkes was led to question an old Pima concerning it.

> When Higgins (the name of the Indian) was shown the figure and told the opening lines of the quotation (from the Spanish narrative), the last clause being withheld, he responded that he knew

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[2] Rudo Ensayo.

of no ancient house in that region which had a ground plan like that indicated in the figure. He was acquainted with a children's game that employed a similar figure traced in the sand. The Pimas, he said, call the figure *Tcuhuki*, the house of Tcuhu, a cultus hero sometimes identified with Moctezuma.

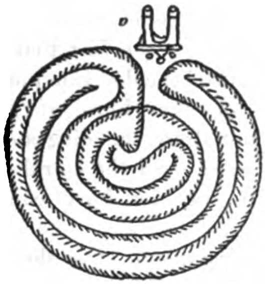

FIG. 1. After Fig. 34, *American Anthropologist*, Vol. IX., 1907, p. 511 (inverted).

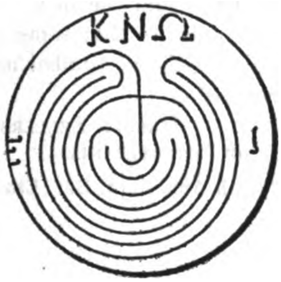

FIG. 2. After a Coin from Cnossus in Crete (200–67 B.C.). (Cat. of Greek Coins in British Museum, Vol. 1887, Plate VI., Fig. 5.)

A search in Russell's work on the Pima Indian[3] and Culin's "Games of the North American Indians"[4] failed to discover a description of such a game. However, Russell did describe a game called *Tculikwikut,* a dart and ring game in which count is kept by means of little stones. These are moved on a diagram made up of a series of small holes in the sand arranged in the form of a whorl arising from a center called *Tcunni Ki* (the council house).

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[3] Twenty-sixth Ann. Rept. Bur. Eth.

According to Russell *Tcuhu* in the mythology of the Pima is Gopher, who dug the spiral hole through which the Pima clans came up from the underworld. From this it seems possible that both Russell and Fewkes were dealing with the same game. Strength is given to this idea by the fact that Dr. Fewkes showed "Higgins" the diagram and the Indian said that it was the House of Tcuhu. The Indian did not draw the diagram. He may have simply recognized the spiral character of the labyrinth and not have considered the details.

With such fragments of evidence and with so many gaps to be filled it would be premature to draw any conclusion as to how this complicated symbol happens to be found in both the old world and the new. The writer publishes this in hopes that some reader will also be familiar with the symbol and can aid in its interpretation.

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[4] Twenty-fourth Ann. Rept. Bur. Eth.

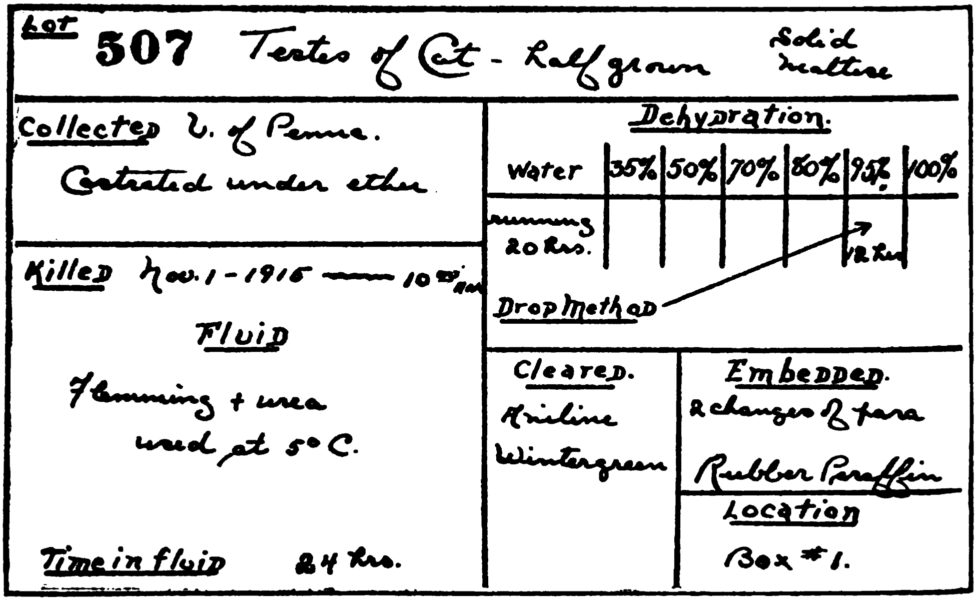
A SYSTEM FOR RECORDING CYTOLOGICAL MATERIAL, SLIDES AND LOCATIONS ON THE SLIDES

The following contribution is offered in full recognition of the fact that many cytologists already have in use excellent methods of recording their material and slides. Indeed many of the most essential details of the present system of recording slides have been taken over from a method in use by McClung, for which privilege the writer acknowledges his indebtedness. The system to be described has stood the test of the writer's use in all particulars and it is offered here in hope that it may serve as a suggestion for cytologists, who as yet have no recording method, on which to base a system serving their own particular needs. If the scheme here outlined is impractical for certain workers this note will at least serve to indicate the requirements that a cytological recording method must meet to be really efficient.

Two sets of cards are used, one to record the gross material and the other the slides and locations on the slides. On the former card (Fig. 1) are all the notes concerning the material from the fresh condition until it is embedded. On this card is to be found:

1. the serial lot number.
2. the material, the animal or plant from which it was taken, the age and other notes of possible interest.
3. place of collection and condition of obtaining the material.
4. dates of fixation.
5. fixing fluid and the temperature the fluid was used at.
6. time in fluid.
7. washing and dehydration.
8. clearing methods used.
9. embedding methods and materials.
10. location of embedded material.

Under "Dehydration" in Fig. 1 the time the material remains in each grade of alcohol is recorded beneath that grade. In case the more recent practice is used (not yet published) of displacing the water with alcohol drop by drop an arrow is drawn, as indicated, to the percentage of alcohol the material is in at the end of the displacement. If the tissue was preserved in 70% alcohol then an arrow would be drawn to "70%" and later when the dehydration is continued a second arrow would be drawn to the grade at the end of the series. The writer embeds his material immediately as in the long run it saves considerable time and tissue in paraffin is much easier to carry about the country than in bottles. The material under each lot number is usually embedded in a petri dish (the lot number on a small piece of paper is embedded with the material). The disk of paraffin after removal from the petri dish is wrapped in wax paper and filed in a 3x5 cardboard filing case behind an index



Lot 507 Testes of Cat - Half grown Solid matter

Collected U. of Penna.
Castrated under ether

Killed Nov. 1 - 1916 —— 10 am

Fluid
Flemming + urea
used at 5° C.

Time in fluid 24 hrs.

Dehydration.

Water	35%	50%	70%	80%	95%	100%
running 20 hrs.					12 hrs	

Drop Method

Cleared.
Aniline
Wintergreen

Embedded.
2 changes of para
Rubber Paraffin

Location
Box #1.

Fig. 1

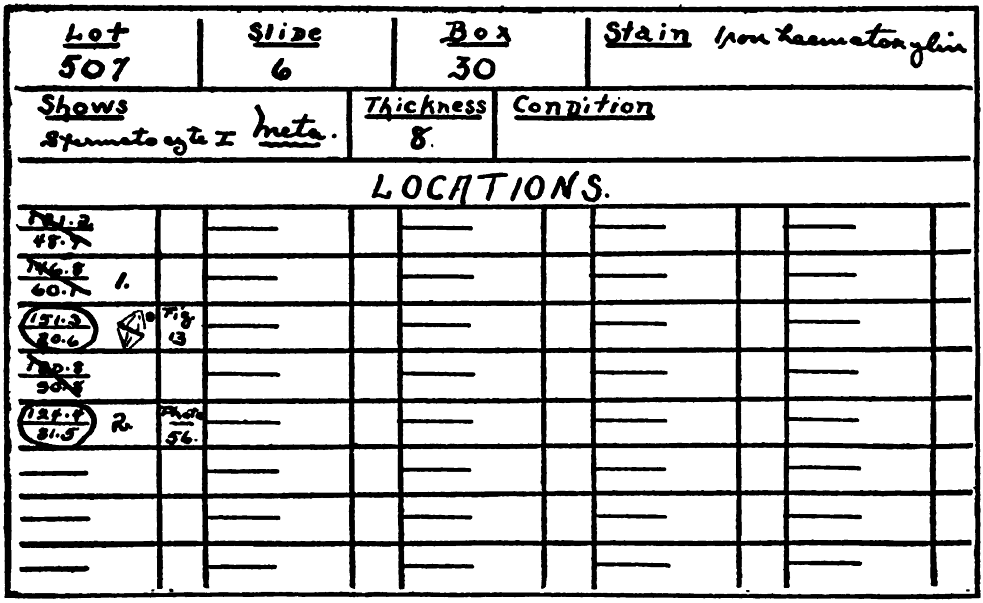
card bearing the lot number. In this way the material is compact and easy to get at.

On the second set of cards is to be found:

1. lot number.
2. slide number.
3. number of box in which slide is located.
4. stain used.
5. what is to be found on the slide.
6. thickness of sections.
7. condition of slide (i. e. good fixation or stain).
8. location of favorable areas on the slide.
9. notes concerning certain locations.
10. areas that have been drawn.
11. areas that have been photographed.
12. areas that have been drawn or photographed and used for publication.

The number is scratched on each slide of the series. If spermatogenesis is being worked upon one card is devoted to a single phase in the process found on a particular slide. Similar phases on other slides have their own cards. This card is labeled as shown in Fig. 2 under "Shows". The same slide may therefore have several cards devoted to it should it show more than a single phase. These cards are filed first behind an index card bearing the lot number and then in numerical order behind index cards bearing the phase name of the particular stage they happen to represent. When the observations do not deal with spermatogenesis then, of course, the cards are classified according to the special need.

As can be seen in Fig. 2 there is a place for forty readings. The right hand reading of the mechanical stage is placed above the short line, the horizontal reading is put beneath it. The slide is first searched with a low power lens and readings of apparently favorable locations are put down. Afterwards these locations are tested with the oil immersion lens and either crossed out or drawn. When the figure is drawn the location is circled as shown in Fig. 2. Any notes that are to be made are indicated by the figures in the space to the right of the readings. These numbers refer to corresponding numbers on the back of the cards under which the notes are written. Small sketches may also be put in these spaces to recall what the reading is of. When the plates have been prepared for publication the figure number is entered in the square opposite the reading. When the cell has been photographed this information is also placed here with the number of the photograph. With these records should the plates be lost or when the original of a figure is to be examined the location on the slide may readily be found.



Lot 507 | Slide 6 | Box 30 | Stain Iron haematoxylin

Shows Spermatocyte I meta. | Thickness 8. | Condition

LOCATIONS.

~~[illegible].2 / 48.7~~		
~~[illegible].8 / 60.7~~ 1.		
151.2 / 20.6		Fig 13
~~[illegible].8 / 90.8~~		
124.4 / 31.5 2.		Photo 56.

Fig. 2

With the records on these cards before him the investigator has all his data well in hand for the preparation of his paper.

Zoological Laboratory, ROBERT T. HANCE.
University of Pennsylvania.

A MINIATURE DARK ROOM FOR USE WITH THE MICROSCOPE

All microscopists prefer to work either at night or in a darkened room. Using the microscope under such conditions does away with the strain to which both the observing and the unused eye are subjected by the side light—i. e., light coming from sources other than through the tube. When working in darkened surroundings the effect is that of looking at a picture on a screen. The image appears brighter and objects become clear that under the usual conditions are scarcely visible.

For several years the writer has been trying to devise some method to control the light perfectly and to do this without necessitating the darkening the whole room. It is desirable that any apparatus for the purpose should weigh little and (for ease in carrying from one place to another), it should be simple to take apart. It should, of course, be adaptable to every condition. For further convenience of the worker definite places should be present in such an apparatus for the usual microscopical accessories—pens, pencils, drawing and memorandum cards and lens paper.

The following description is of a miniature dark room for use with the microscope fulfilling these requirements. It was designed and made by the writer last fall and, after a year's use, he has found it to be exceedingly practical in eliminating all the strain that results when the eye is unshielded. In this darkened enclosure the eye not in use is at perfect rest. Moreover for drawing the light may be controlled so that it is possible always to have light of the same intensity directed on the drawing paper.

Description

Figure 1.

A. Base—¼-inch white pine 12x18 inches with a binder of the same wood across each end to prevent warping.

B. Uprights—dowel sticks 1 inch in diameter cut to 18 inches in length.

C-C′. Rods—common telescoping curtain rods. Each of the rods C′ is cut 8 inches from the end that ordinarily would be used to fasten it to the window. C is formed of the remainder, of the part between the ends.

D. Wire—a piece of annealed wire ⅛ inch in diameter about 4½ feet long bent as shown.

To assemble:—one two inch screw fastens each upright to the base. The upright on the right can be seen to have two angle irons aiding in its support but this is only necessary when the fan is added. Holes are drilled in both uprights to correspond to the diameter of C which is inserted in them. The rods C′ are attached by one end to the tops of the uprights by a screw through the eyelet in the rod. Through the eyelet at the opposite end a small rod is passed as shown to prevent the curtains from slipping off. The wire D is fastened to the outer sides of the uprights by means of a single round head screw passed through each flattened end. All the wood and metal work is painted a dead black.

For many valuable suggestions on the design of the curtains and for the excellence of their construction I am indebted to my mother. (See figure 2).

The curtains suspended from the rods C and C′ are in four parts, all overlapping each other and fastening together with spring snaps. They are made of the heaviest grade of black sateen doubled. On the right hand curtain are pockets for pencils and cards. On the left side is a pocket for lens paper. The pocket is provided with a flap to exclude the dust. The upper curtain carried on the wire D is of single thickness. The central curtain is in two parts so that they may be separated to permit light to fall on the drawing board. The left hand curtain of the central set has a rectangle 1 inch wide by 5 inches high cut from the center of the basal portion. Across the top of this aperture is stitched a flap of double thickness, 3½ inches wide by 5½ inches in length. To one corner of the loose end of the flap is attached a tape which passes around the tube of the microscope and fastens to the other corner of the flap by means of a spring snap.

With the microscope surrounded by these curtains it is impossible to read the figures on the mechanical stage and so the small light (fig. 1 E) was installed. This can be adjusted by means of sliding rods locked with winged nuts to hang directly over the stage. The lamp arm is attached to the right hand upright by means of a collar made of two pieces of brass stripping fastened on either side of the pillar with a thumb screw. The lamp is a small tungsten bulb set in a porcelain socket. The shade or reflector, shown in the photograph, was taken from an old tubular flash light. A small three-cell pocket battery furnishes the current which is

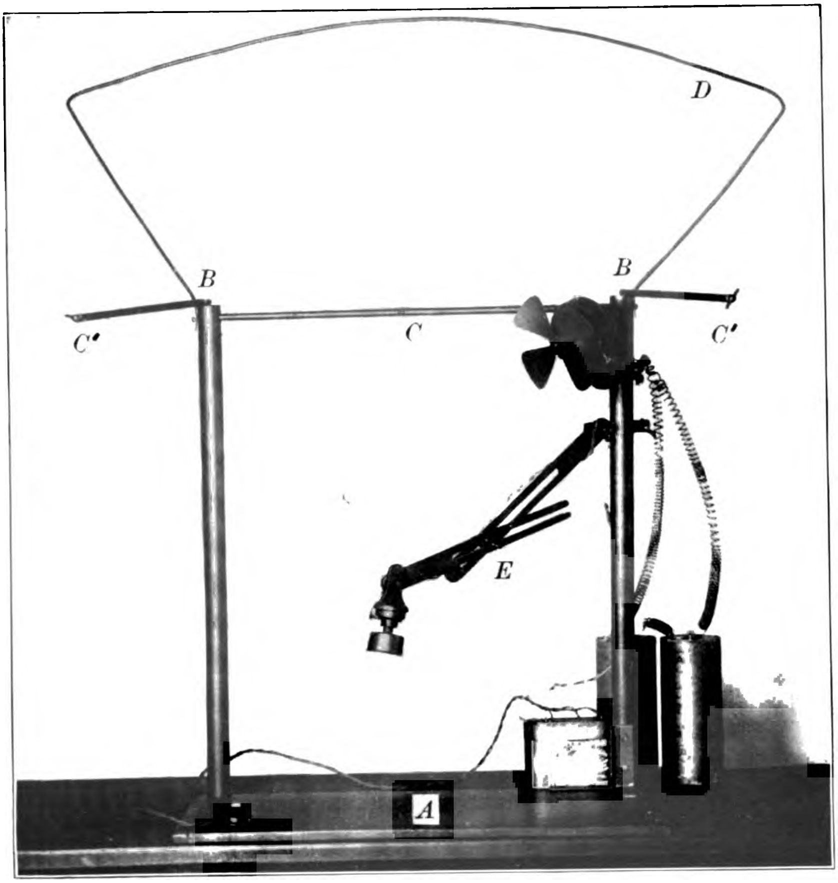


Plate XII

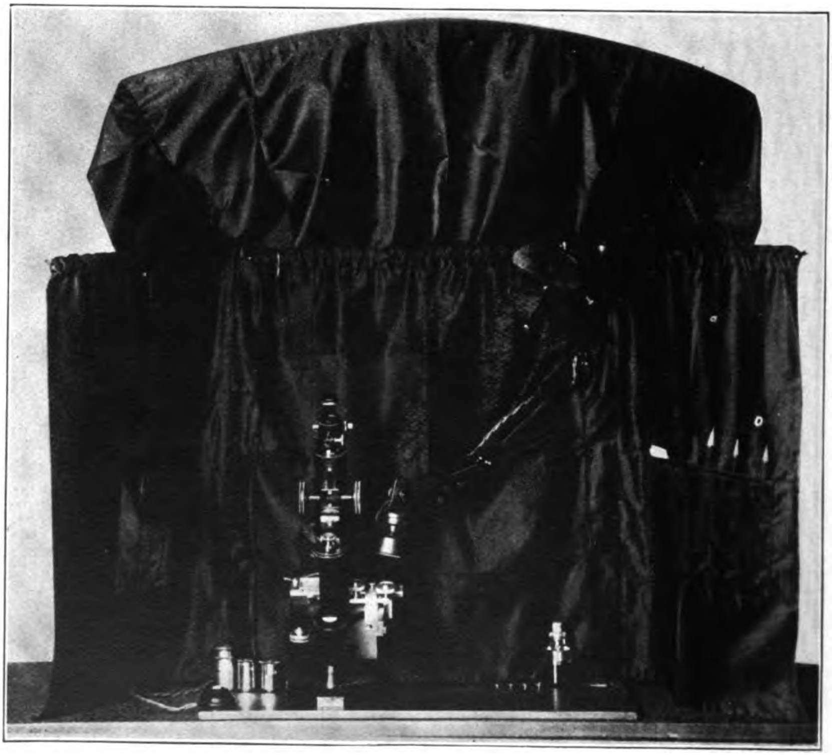


PLATE XIII

controlled by a push button at the left of the microscope. The same battery has lasted for very nearly a year now without visible signs of weakening.

The fan shown in both photographs is a toy motor equipped with a 4½ inch blade. The motor is operated on two dry cells. It is fastened to a wooden base that is inserted in a slot in the upright and clamped tight by means of a winged nut. This fastening permits the fan to be tilted up and down while the single screw securing the fan to the base allows a left and right rotation. The air current may thus be directed on any spot desired.

Operation

For microscope illumination with this dark room a concentrated filament mazda frosted globe is used. This globe is placed behind the slit in the central curtain and the microscope is put in position on the opposite side. The flap covering the slit is then snapped about the tube of the microscope just above the nose piece. The slit through which the light comes is so narrow that the stage of the microscope effectively shields the eye from the light coming through the lower part of the slit while the flap takes care of all other dispersion.

In the average room having windows on only one wall the side curtains can be left wide apart. In places where the worker is almost surrounded by windows it is of advantage to draw the side curtains so close that there is just room for the observer's head to enter. The telescoping rods supporting the side curtains permit these to be narrowed or widened to suit the circumstances. The top curtain works to or from the microscopist and is frequently convenient in cutting out the light from the upper parts of the windows.

Light on the drawing paper is obtained by separating the lower portions of the central curtains from each other and fastening them back. The bulb illuminating the microscope then throws its light over the right hand side of the base. A constant intensity of illumination is in this way assured.

The fan is a luxury—possibly an unnecessary one, but in very warm weather or on days when a few flies persist in maintaining their position at all hazards on top of the writer's head he has not been at all skeptical as to whether the luxury was unnecessary or not.

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NOTES ON HANDLING PROTOZOA IN PURE LINE WORK

During the past year the writer has been engaged in experiments on the inheritance of extra contractile vacuoles in a new race of Paramœcium and has worked out some methods of technique that have so facilitated his work that he is led to publish them in hope that they may be of benefit to others.

Maintaining pure cultures.—The greatest care is necessary to prevent pure line cultures from becoming mixed with others. Even with labelled pipettes accidents may occur. The scheme shown in the cut was recently devised and has proved most convenient. A piece of soft brass wire is shaped about some round object of a diameter slightly larger than a pipette and is held by several twists. Then the long ends of the wire are bent around the culture jar and again fastened by twisting the ends. In the jars used by the writer (humidors bought at any five and ten cent store) there is a convenient groove near the top into which the wire fits nicely. When finished the small circle protrudes from the jar and into

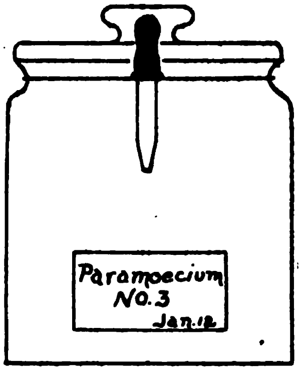

FIG. 1

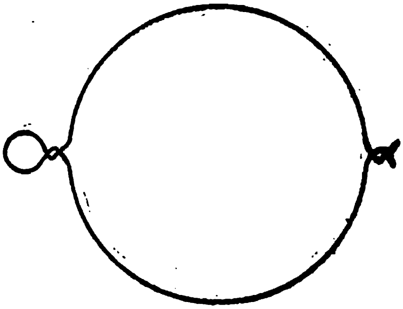

FIG. 2

this ring the pipette is dropped giving the appearance of Fig. 1. With this method pipettes are always at hand and there is no danger of mixing the lines by transfering animals (clinging to the walls of a pipette) from one culture to another.

Preparation of watch glasses.—Syracuse watch glasses have been used for single individuals throughout the writer's work and considerable difficulty was experienced at first in locating animals which were close to the edge of the container. They frequently found their way there as the fluid had a tendency to spread evenly over the surface of the watch glass. Several methods were tried to correct this tendency of the culture medium to spread over the bottom but the best one was hit upon accidentally. There was a trace of paraffin in a pan in which the glasses were being sterilized one day and this coated the glasses imperciptibly but sufficiently to give the liquid no hold on the glass. In vessels treated in this way the surface tension of the medium tends to draw it into a spherical mass. Should the liquid roll to the edge of the glass where the animals would be hidden from view, it is easily rolled out again by tilting the glass and none of the animals in the drop are left behind. When animals are being kept in very small drops of water the writer has placed as many as twenty individual drops of liquid containing protozoa in a single watch glass and they have not run together. The surface tension of the liquid draws it up, when on a paraffined surface, until it gives a very fair picture of a drop of mercury. Furthermore, being contracted to the smallest area possible there is less evaporation than when the same amount of fluid is spread out and the chances of losing a valuable specimen through drying are very much less. The writer's practice is to use a piece of paraffin about the size of a pea to a quart of water. This will be sufficient for a surprising number of watch glasses. When the sterilized glasses are removed they are wiped while hot and polished. No paraffin is visible although a faint trace of it can be felt.

Making Pipettes.—In this work the most useful pipette has been found to be one that has a short but very fine tip. The usual methods of drawing them tend to make a pipette with a rather long tip as the glass tube has been heated for some distance equally along its length. To cut down the area heated the tube to be drawn is placed transversely across a fish tail flame, which heats equally an area certainly not more than a quarter of an inch at found their way there as the fluid had a tendency to spread evenly the most, and the tube is pulled with considerable force when the glass is just commencing to melt. Several trials will show the best time to start pulling. This method gives pipettes with very fine tips not more than from three-quarters to one and one-half inches long.

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NOTES ON EMBEDDING IN PARAFFIN

When embedding very small objects, such as insect larvæ or small flowers or anthers, in paraffin it is most convenient to orient them one behind the other. This method allows a single block to be made of from three or four to a dozen pieces of tissue and these may be cut in one ribbon. This obviously eliminates a great deal of the labor involved in making a block of each separate object, cementing it to the holder, trimming it and adjusting the microtome each time. In the ribbon it is easy to see where one piece of tissue ends and the other begins as there is usually several blank sections of paraffin between them. It is relatively simple to arrange the tissue in line under a carbon bulb with warm needles but a difficulty is met with when an attempt is made to place the paraffin mold in water for cooling. The material is shaken from position and must be reoriented. This had been overcome in the following way. A watch glass is used as a mold for embedding small objects and a petri dish is convenient for larger tissue. When the tissue is ready to be embedded the dish is heated to the melting point of the paraffin under the electric bulb. It is then placed in a crystallization dish with two slides beneath it to prevent it from touching the bottom of the container. Paraffin is then poured into the small dish and the objects oriented as desired the heat of the electric bulb keeping the paraffin melted. Then the light is turned off and cold water is poured into the crystallization dish. Since the dish containing the paraffin is raised from the bottom the water flows under it and soon solidifies the paraffin in the lower part of the dish which consequently holds the objects fast. As soon as a surface film is formed enough water can be added to cover the embedding mold to complete the hardening of the paraffin.

In petri dishes or watch glasses the bottom is practically flat and true and the tissue is allowed to sink to the bottom. When the tissue is cut out as a block the part that rested against the bottom makes one of the two parallel sides and requires little or no trimming.

When a number of pieces of tissue or a number of series (as described above) are embedded in one disk of paraffin it is dangerous to attempt to separate them with a knife as one can never be sure of the direction the crack in the paraffin will take. I have found that a hand scroll saw or coping saw (which may be purchased for ten to twenty-five cents) does admirably for cutting a block of tissue from the main disk. A hot wire is used by some but is not nearly so convenient nor so accurate as the saw. The use of the saw permits many more pieces to be placed in the same space as no care need be taken to have well defined pathways for the paraffin to split along as is necessary when a knife is used for separating the pieces.

Robert T. Hance.

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June. 1916

A SIMPLE PARAFFIN RIBBON WINDER

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TWO FIGURES

Several years ago McClung designed a cylinder revolving on an axle borne on two standards for the purpose of picking up the paraffin ribbon as it left the knife of the microtome and winding it lightly around the drum. The value of such an apparatus is obvious. Long serial sections may be wound entire onto the cylinder doing away with the danger of scattering the ribbons by a sudden puff of wind as sometimes happens when they are laid on paper in the usual way. It further does away with the necessity of piecing portions of ribbons together on the slide since a continuous ribbon allows strips of exact length to be cut.

The writer designed a machine for the same purpose (described in the Trans. Amer. Mic. Soc., December, 1913) to be used with the Leitz base sledge microtome which carries the knife much higher than most cutting machines. It is not nearly so simple to make, however, as the one to be described in this paper.

McClung's model has been adopted by one of the American microscope manufacturers. Since the device is so exceedingly useful and the price of the machine on the market is beyond the limits of the average student's resources, the writer recently made a ribbon winder that can be built in half an hour and costs approximately eleven cents. It is so simple that every student of histology or embryology can make one for his or her own use.

DESCRIPTION

The dimensions may vary with the materials at hand and the measurements of the machine pictured will be given merely to present a general idea of the size relations.

Base. 8 inches by $3\frac{1}{2}$ inches, $\frac{7}{8}$ inch thick.

Uprights. A one inch dowel stick was used although a broom handle or even a flat board would serve. A quarter inch hole was drilled through the center of the dowel which was then cut across the diameter of the hole. This gave two sticks with a semicircular groove in one end. Each of these sticks was now cut to an exact length (in this case $3\frac{7}{8}$ inches) and fitted into holes bored in the base the centers of which were $5\frac{3}{4}$ inches apart.

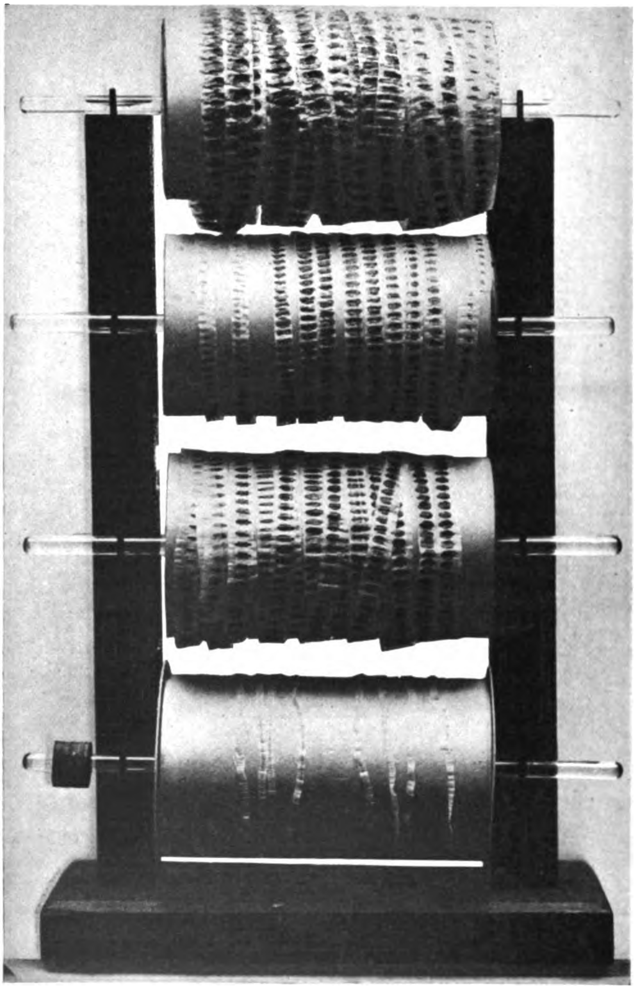

Figure 1

Cylinder. The cylinder was made of 2½ inch cardboard mailing tube. I have made them of smaller sizes which work just as well. A twenty inch length of this tubing may be purchased for five cents and then cut into five lengths with a hand scroll saw or coping saw. Thin, flat corks, 2½ inches in diameter, are bored to take a glass rod and are then fitted into the ends of the tube. A glass rod eight inches long is thrust through the holes. This forms the axle which fits into the semicircular grooves in the uprights as shown in figure 1. The tube is finally wrapped with paper to give a smooth surface and the apparatus is finished.

Some workers prefer the roller to have flanges on either end. These may be easily made by gluing disks of heavy cardboard against the ends of the drum as shown on the lowest cylinder in figure 2. On the same drum is shown a cork fitted to the axle on one side which the worker can add should the glass rod not give sufficient purchase for easy turning.

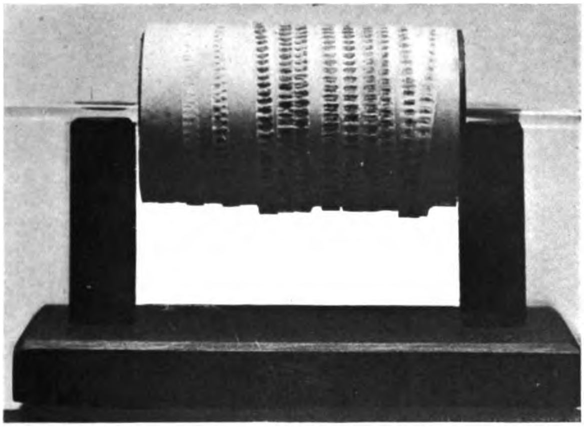

Figure 2

Instead of a glass rod for the axle a quarter inch dowel might be used. Dr. McClung has suggested that a wooden mailing tube would serve for the cylinder and might even cost less than the cylinder with cork ends described above. Since these mailing tubes are turned up on a lathe the ends show the centering marks and make it easier to locate and drill the center for the axle than with the corks.

When in use it is necessary to slide the apparatus side ways so that the ribbon is wound spirally. That the mechanism may move smoothly three so-called domes of silence (such as are used on chairs) may be fastened to the bottom. These, however, are not absolutely essential.

The ribbon may be wound over and over itself without danger of sticking. The ribbon on the top cylinder of figure 2 is between 40 and 50 feet long and is unbroken. It may be allowed to remain on the drum in perfect safety until the worker has time to mount it. Recently I have allowed a ribbon to remain on the cylinder uncovered and subjected to the usual variations in a room for four weeks and at the end of the time mounted the ribbon without trouble and without loss of a section.

A storage rack for the cylinders. When a worker has several blocks of material to cut it is of convenience to do them all at one time. For this purpose a number of drums are necessary and to take care of the drums a storage rack was made as shown in figure 2. The dimensions are the same as for the last base described with the exception of the uprights which are twelve inches high. When one cylinder is filled it is placed on the hooks fastened into the upright as shown in the photograph and a fresh cylinder is placed on the working base.

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February, 1917

A DEVICE TO INCREASE THE EFFICIENCY AND EASE OF MANIPULATION OF THE FINE ADJUSTMENT OF THE MICROSCOPE

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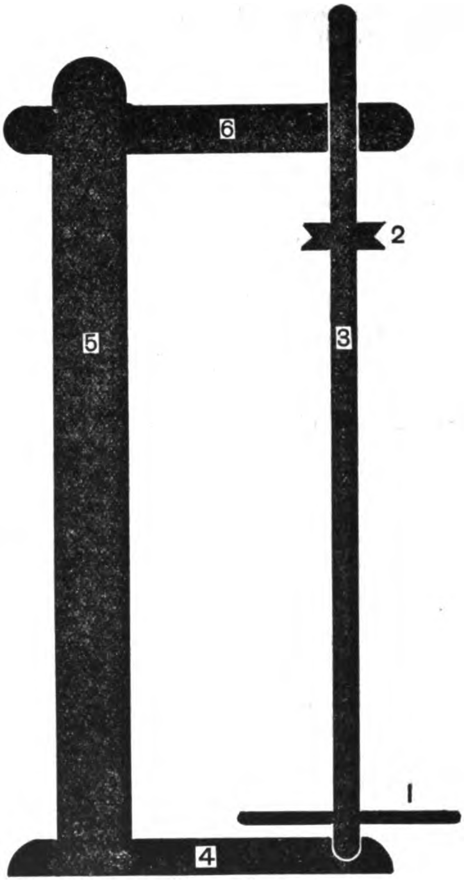
ONE FIGURE

The use of high powered microscopic lenses requires the hand to be constantly on the fine adjustment. When the study is continued for several hours at a time the hand and arm doing the focusing, even though the latter is supported at the elbow on the table or chair arm, become extremely fatigued. To obviate this strain the apparatus figured below was devised. Although the role it was originally intended to fill was merely to facilitate the use of the fine adjustment, it has, since its installment, not only been of aid in this way but has greatly diminished several annoying features that are frequently connected with high power work. For this reason it seemed worth while to bring the attachment to the attention of microscopists.

The device as figured is for use with microscopes which have the more common 'top' fine adjustment although it would be a simple matter to construct a similar apparatus for use with the side wheel type. No dimensions are given, as these must be controlled by the height of the particular microscope the attachment is intended to be used with. The illustration, which is a side view, is just one-half the size of the one I have in use. The entire apparatus is made of wood and it is simpler to use dowel sticks of various diameters for the pillar (*5*), the arm (*6*), and the shaft (*3*), although other material could be used satisfactorily. The focusing wheel (*1*) is a disc cut from lumber about one-eighth of an inch thick. It is of advantage to have this wheel large (two to two and one-half inches) and thin, so that it may be easily gripped and turned with either the thumb or fingers. The edge of this disc is rounded and notches are filed into it at intervals of about one-quarter of an inch to prevent the fingers gripping it from slipping. The grooved wheel (*2*) is made by gluing together two discs about one inch in diameter (cut from thin lumber) whose adjacent rims have been beveled. It is well to have the channel or groove rather deep so as to prevent the belt from jumping when the microscope is inclined.

Care, of course, must be taken to center the hole in the arm (*6*) through which the shaft (*3*) passes and the socket in the base (*4*) into which the end of the shaft slips. Smoothness of action is obtained by soaking that part of the shaft that is in contact with the arm, and the hole in the arm through which the shaft passes, with paraffin. The socket in the base is filled with paraffin so that the shaft is resting in a



tightly packed bed of this material. I am indebted to Dr. C. E. McClung for suggesting this method of lubrication, which results in a very soft, smooth movement.

In use the apparatus is screwed to the table (in my case to the drawing board on which the microscope rests) with the pillar (*5*) away from the observer. A belt composed of string which will not stretch (such as braided fish line) is passed around the pulley (*2*) and the focusing wheel of the microscope. The microscope may be used in any position. The focusing arm lies flat on the arm of the chair or the table and the focusing wheel (*1*) is within easy access.

Since the pulley (*2*) is made smaller than the fine adjustment wheel of the microscope the sensitiveness of focus is increased. The delicate focusing of high powers has been found to be much steadier with this attachment. The annoying feature of the field under observation being a trifle displaced while drawing, due to the hand on the focusing wheel jarring the microscope (however little), is largely eliminated with the use of the device described as it is now unnecessary to directly touch any part of the microscope.

[*Reprinted from* SCIENCE, *N. S., Vol. XLV., No. 1157, Pages 221-222, March 2, 1917*]

GOLDFISH AS EMBRYOLOGICAL MATERIAL

FEW laboratories have at their disposal a constant supply of material for the study of living embryology. Frog and snail eggs are used occasionally, but the supply is uncertain and sometimes difficult to obtain in the right stages, and furthermore, these ova are not particularly favorable for study. While engaged in an investigation on the genetics of goldfish the availability of the eggs of this animal for studies on the living embryo became evident and led to this note.

Goldfish are readily obtained in almost every locality and are thoroughly normal in environments in which few wild fish could exist. They breed in tanks containing not more than fifteen or twenty gallons of water and consequently require no elaborate or extensive equipment. Cypress boxes a foot or more square and three feet long make excellent breeding tanks.

A half dozen pairs or less will supply ample material for class use. It is not necessary to have as many males as females. It is well to obtain fish at least four or more inches long, as the larger fish are more certain to mate and are much more prolific. It is impossible to distinguish the sexes except as the breeding season approaches, when the sides of the female become distended through the growth of the ovaries and small spiny projections appear on the operculum and the anterior edge of the pectoral fins of the male.

One or two months before the breeding season begins it is advisable to feed the adults small quantities of beef, liver, mosquito larvæ

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March, 1917

THE SOMATIC MITOSES OF THE MOSQUITO CULEX PIPIENS

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TWO PLATES

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INTRODUCTION

The great mass of cytological work has been done on the germ cells, partly because of the abundance of mitotic stages found there, partly because of the greater ease of manipulating this tissue, but largely because of the great theoretical importance of the processes here visible. Beyond a few general sorties into the field of somatic cell division, which determined that the body chromosomes were usually present in the same number as in the reproductive cells, and were, in some instances, at least, similar morphologically, little has been done in the way of thoroughly working out the mitotic mechanism from prophase to telophase.

The ideal investigation into somatic cytology would follow the chromosomes of the fertilized egg as it evolved into the organism and as the cells differentiated into specialized tissues, but unfortunately material favorable for the study of development is usually hopeless for cytological work, and the reverse is true. Some success which I have had with mosquito eggs leads me to believe that improved technique would make this insect a favorable form for such a study, but until such a technique has been developed, the study of the division figures found in the larvae of this form will prove, I think, both interesting and valuable. Because of an interest in somatic chromosomes and because of the contradictory reports that have been published on Culex this animal was selected for study. The results are complementary to Mr. Whiting's thesis on the spermatogensis.

In this paper only what may be termed the normal somatic divisions will be considered, as Miss Holt has made a study of the multiple complexes.

It is a pleasure to take this opportunity of thanking Dr. McClung for the many facilities he has placed at my disposal and for his constant interest and advice. Discussions with my fellow workers Dr. Caroline Holt and Dr. P. W. Whiting have been of great assistance to me.

TECHNIQUE

An abundance of material was found in tubs of water standing in the greenhouses on the Campus. Larvae and pupae have been fixed, but only the larvae show any number of somatic divisions. The question of the method of fixation is an exceedingly important one, as the reasons for the various conclusions reached by other investigators of Culex chromosomes are quite evident from the results obtained with various fixatives. Eight different killing fluids were tried, each at three temperatures, 5°C., room temperature and 38°C. Of these the best has proved to be Flemming's solution to which had been added a little urea and used at room temperature. The urea was added to increase the rapidity of penetration. Another fluid which gave good results for some stages was what is designated in this Laboratory as P. F. A. No. 3 which was used at 38°C. The formula for this fluid is as follows.

	cc.
Picric acid saturated	75
Formalin	15
Glacial Acetic acid	10

Flemming's solution gave much the more accurate results particularly on the earlier stages. For perfect fixation with this fluid, however, the larvae must be teased finely in the solution or else the chitinous covering will prevent penetration. P. F. A. No 3 gives excellent results on the metaphase chromosomes but the early stages of the chromosomes are very likely to be wholly or partially fused, giving a totally erroneous impression of the actual conditions. When larvae were fixed in this killing fluid the head and thorax were severed from the abdomen and before placing in the warm fluid. Smears of the thorax were made and fixed in Flemming's solution but although thirty animals were prepared in this way no division figures were found. The general fixation was very good.

The material was dehydrated by allowing 95 per cent alcohol to displace the killing fluid drop by drop, until the fixative was entirely eliminated and the tissue stood in 95 per cent alcohol. When Flemming was used the material was first washed in water and then dehydrated in the same way. It was found unnecessary to remove the picric acid completely, as is usually advised following a picric acid fixative. The tissue fixed in P. F. A. No. 3 was cleared in aniline oil and the Flemming material was cleared in cedar oil. Either of these oils was followed with xylol, imbedded in paraffin and cut in sections six micra thick. In imbedding a number of thoraces or bodies the pieces were oriented one behind the other, which simplified the cutting materially (Hance, '15). When finely teased tissue was being prepared for cutting, the material in xylol was poured into a paper box or boat and the xylol was drained off through the paper, leaving the tissue massed at one end of the boat. The paraffin was poured into this paper form and the box was placed in melted paraffin. In this way it was easy to retain the finely divided tissue. Iron alum-haematoxylin was used exclusively in staining.

The methods of technique practiced here gave results consistent with others obtained in this Laboratory and are part of a number of experiments, employing the same reagents, under similar conditions.

OBSERVATIONS

Miss Taylor ('15) states that she had trouble in finding somatic mitoses, but I have had very little difficulty in locating active cells. The cells of the general tissues of the body are usually in a resting condition, but the limb buds in the thoracic region and the nervous tissue are frequently full of division figures. It is an interesting fact that the somatic divisions show some indication of rhythms of cell activity. The tissue may be entirely inactive or, when active, a large number of cells are taking part, and the majority of these cells will be in approximately the same stage of division.

I have never found any evidence of synizesis in either germ or somatic tissue, except in cases where the material was obviously very poorly fixed. While it seems evident that there must be some physiological (or probably only physical) peculiarity about the stage in which synizesis is observed which permits it to act differently to the other stages, nevertheless, I believe that the actual appearance of synizesis in fixed material to be an artefact.

Although most of the drawings have been made from mitoses found in the nervous tissue, similar stages have been found in most of the tissues of the body. These include nervous tissue, limb buds, intestine, epithelium, ovarian tissue and Malphigian tubules.

Prophase. The somatic cell in the resting or early prophase condition possesses a nucleolus. This disappears as the chromosome filaments appear. The earliest prophase stages I have found in active tissue are shown in figures 2 to 16. As can be seen there are three pairs of threads (two long pairs and one shorter pair) present, all more or less closely associated. The importance of the proper fixation in these stages can not be overemphasized as I was misled for some time by finding (in the

P. F. A. No. 3 fixed material) only three threads with splits occasionally appearing at the ends of the threads. It is easy to understand how, unless the fluid reached the cell almost immediately, the delicate splits or lines of separation would be completely or partially obliterated. Such a case is shown in figure 1 where only three threads appear. It is very interesting to note the orientation of the three pairs of chromatin threads in the prophase. If the plates will be referred to it will be seen that the apices of the three V's in each cell (the point of spindle fiber attachment) are directed toward the same pole, suggesting the position in which the chromosomes lie when last seen in the anaphase. This condition is very suggestive from the point of view of the individuality hypothesis. As the prophases approach metaphase the paired chromatin threads seem to separate and gradually lose the characteristic prophase orientation. The beginning of this process is illustrated in figures 13 to 16.

I am unable to account, at present, for the size variation which occurs between the chromosomes of various cells. This will be apparent chiefly in the drawings of the prophase figures. Figure 7 is of an especially large cell. It will be noticed that the size relationship between the chromosomes of the same cell is apparently the same in all cases.

Metaphase. When the arrangement of the prophase is completely lost, the threads have shortened and thickened, have separated rather widely and the characteristic metaphase plate stage appears, figures 17 to 24, 26 to 30. This stage Whiting has designated as stage A. The chromosomes undergo another rearrangement and the three pairs line up side by side preparatory to division (Whiting's stage B), figures 25 and 31. A good side view of the spindle with the chromosomes distinctly visible is difficult to find but figures 32, 33 and 34 were drawn from clear cells in which the plates were slightly tilted permitting the six chromosomes on the spindle, each split preparatory to division, to be seen. Spindle fibers are visible but their actual attachment to the chromosomes is not evident. Judging from the anaphase figures, however, (fig. 37*a*) it seems evident that the fiber is attached to the apex of the V.

Anaphase. Clear anaphase figures are exceedingly difficult to find and the chromosomes are likely to be fused as in figures 38 and 39, giving a wrong impression of the real conditions. It would appear from these figures that three chromosomes are drawn to either pole, whereas in reality the drawings represent six chromosomes, as shown in figures 35, 36 and 37, the apices of whose V's have fused. In figures 35 and 36 six chromosomes are to be seen being drawn to either pole. Though these appear as rods they are in reality Vs, one arm of which is under the arm figured. In these cases the V-nature of the chromosomes can be determined by focusing. This condition is shown in figure 37*a* where one of the pairs has been twisted into the plane of the section. Figure 40 shows six chromosomes in anaphase from a polar view. There seems to be a tendency for the anaphase chromosomes of the somatic cells to turn slightly at right angles to the plane in which they separate, as Whiting has reported for the germ cells. In figure 36 the ends of the chromosomes are all tilted vertically to the plane of the paper. Whether this is due merely to the angle at which the arm of a V might lie or is a real change of axis, I am not certain.

Artefacts. In all stages of mitosis in poorly fixed material are to be found expressions of the faulty technique. A fairly complete gradation of fixation may be found which helps to indicate how other investigators (Seiler and Taylor) may have been led astray in their studies of the chromosomes of Culex. In cells so exceedingly small as these the minute separations would be very quickly obliterated if they were not immediately preserved by the killing agent. The fusion of the chromatin elements is a very common occurrence unless great care is taken to preserve the tissue properly, as I have shown in a paper to be published shortly on mammalian technique. (Hance '17.)

DISCUSSION OF RESULTS AND OF THE LITERATURE

The above account of the somatic mitoses has shown that there are six chromosomes in the body cells, corresponding in size and position to the six chromosomes of the spermatogonia found by Metz ('16) and Whiting (in this journal). In describing the ovogonial and spermatogonial chromsomes Miss Stevens ('10) has reported much the same cytological conditions in Culex pipiens as have been found by Metz and Whiting. "Each of the six chromosomes divides longitudinally, and the pairing of the daughter chromosomes probably occurs in the telophase, for very early prophases show the chromosomes paired and twisted together, forming three spireme threads which gradually shorten and separate for mitosis."

Miss Taylor reports three chromosomes for a number of somatic cells but fails to find any cells showing six. She figures a split appearing at the ends of some of the chromosomes, which condition I find in poorly fixed material. The split indicates the beginning of the line along which two chromosomes have fused. Her failure to find six may be due to imperfect fixation, as may be judged from her figures. The importance of proper fixation has already been emphasized.

In the case of Culex pipiens it is evident that when a spermatozoa with three chromosomes unites with an egg with three chromosomes the somatic number of six is produced. The comparatively little work which has been done on somatic mitosis has tended to show that the behavior of the somatic chromosomes may not always be the same as that of those of the germ cells. (Hoy '16.) By this I do not mean that the differences which seem to exist need in any way interfere with the current cytological hypotheses, although other writers seem to think so. It is quite evident that in Culex pipiens there is no difference between the two groups of cells. It will be of interest in this connection to review the chief literature dealing with the body chromosomes. A discussion of the results will not be attempted at present.

The breaking up of the chromosomes in the somatic cells of Ascaris is too well known to need further discussion.

In Morril's work ('10) the drawings of the somatic chromosomes in Archimerus alteratus, Anasa tristis and Chalinidea vittigera are generally larger and longer than those of the reproductive cells. The relative proportions of the two sets of chromosomes appear to be the same, however. In an Anasa tristis embryo possessing twenty-two chromosomes he found a cell with twenty-three chromosomes and in a twenty-one chromosome Chalinidea embryo a twenty-two chromosome cell was found. Morrill noted this difference in size and stated that the chromosomes of the embryo started out long and shortened as the embryo became older.

In Seiler's paper ('14-'15) on the cytology of Lepidotera some figures of somatic chromosomes are quite noticeably larger than are the spermatogonial chromosomes. The number is the same.

Wieman ('13), in his very clear somatic figures taken from a human embryo, found the number of chromosomes to vary, and he pointed out that, "it is also known that the somatic mitoses do not always show a number identical with the premeiotic one." He reviews other similar cases which I have omitted.

In a paper on the Wandering Jew ('15) I called attention to the morphological difference between the somatic and the germinal chromosomes; the former being much more elongated. This may not be an entirely fair comparison, however, as the pollen eventually aborts and the behavior of the pollen chromosomes is pathological. In a study I have been making of the Oenothera mutant scintillans the somatic chromosomes have a wide range of number (from 15 to 21) in the cells of the same plant. It has been possible to show in this case, by special methods developed for the analysis of the chromosomes, that the 'extra' chromosomes are the product or result of fragmentation of the longer chromosomes, that the fragments divide regularly and that the total length of the chromosomes in the cells possessing extra chromosomes is the same as that of the type group. The relation of the somatic to the germinal chromosomes has been conclusively shown.

In his book, Heredity and Environment, Conklin ('15) has made a statement that would seem to sum up the suggestions of Wieman and which expresses my own belief. "Differentiations of cells are not due to the differentiations of their nuclei, but rather the reverse is true; such differentiations of nuclei as occur are due to differentiations of the cytoplasm in which they lie.

Nevertheless differentiations do not take place in the absence of nuclear material, and it seems probable that the interaction of nucleus and cytoplasm is necessary to the formation of the new cytoplasmic substances which appear in the course of development."

SUMMARY

1. Three pairs of chromatin threads appear in the prophase. The threads are V-shaped and the apices of the V's are directed toward the same pole suggesting the anaphase position (figs. 2 to 16).
2. In the metaphase the threads have shortened and thickened and have become more widely separated. There are three very definite pairs or four long and two shorter chromosomes (figs. 17 to 31).
3. Before the division begins the position of the chromosomes is altered once again and the chromosomes come to lie side by side (figs. 25 and 31).
4. The spindle fibers are apparently attached to the apices of the V's.
5. The somatic chromosome number is six, as is that of the spermatogonia and ovogonia.
6. The number is the same for all the tissue studied. These include nervous tissue, limb buds, intestine, epithelium, ovarian tissue and Malphigian tubules.
7. The importance of proper fixation has been emphasized.

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DESCRIPTION OF PLATES

All the figures are reproduced at a magnification of approximately 4500 ×. All the drawings were made from the Flemming preparations, except figure 29, which was taken from tissue fixed in P. F. A. No. 3. The drawings are all taken from nerve tissue except figure 29, which is from a limb bud.

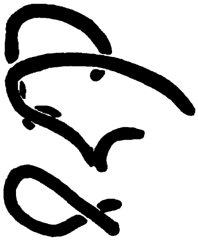
PLATE 1

EXPLANATION OF FIGURES

1 Prophase showing only three threads. This cell is the product of poor fixation.

3 to 16 Prophase stages.

17 to 20 Metaphase stages, polar view.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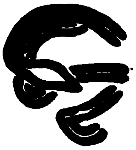
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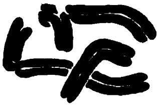
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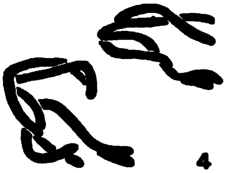
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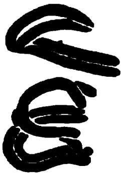
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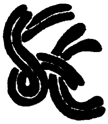
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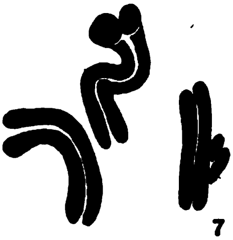
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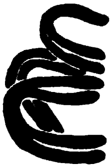
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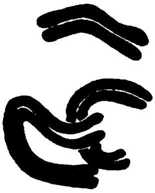
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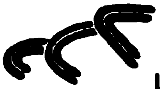
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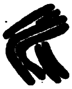
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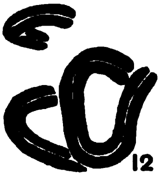
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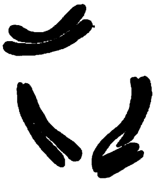
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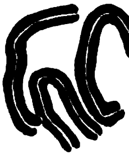
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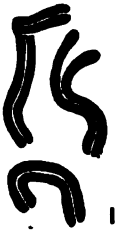
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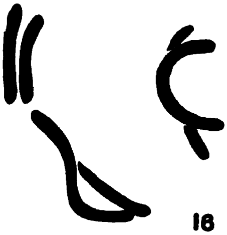
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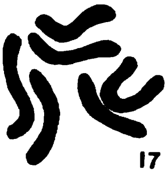
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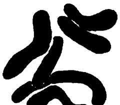
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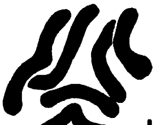
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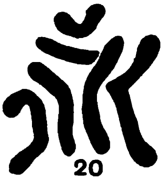
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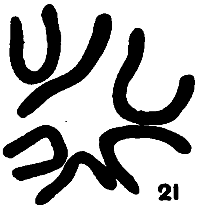
PLATE 2

EXPLANATION OF FIGURES

21 to 31 Metaphase stages, polar view.
32 to 34 Metaphase stages, side view.
35 to 37 Anaphase stages, side view.
38 and 39 Poorly fixed anaphases.
40 Anaphase stage, polar view.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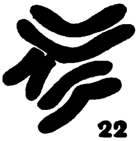
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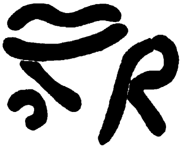
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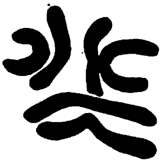
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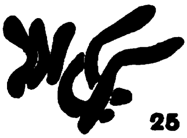
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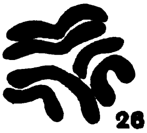
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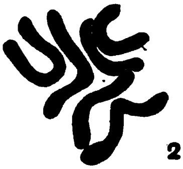
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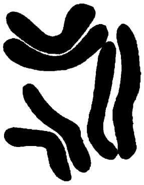
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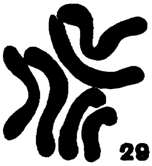
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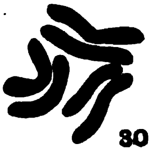
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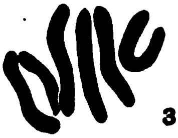
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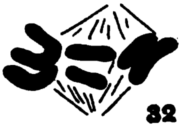
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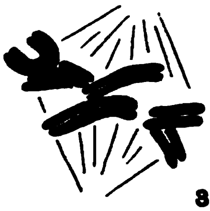
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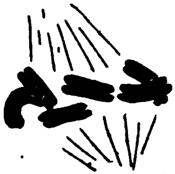
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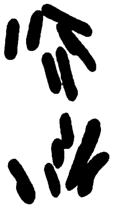
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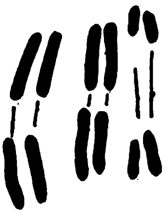
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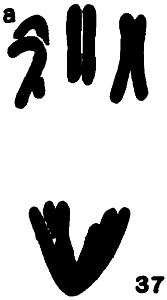

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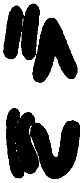
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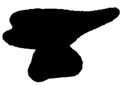
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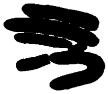

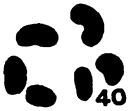
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THE FIXATION OF MAMMALIAN CHROMOSOMES

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Zoological Laboratory, University of Pennsylvania

TWO TEXT FIGURES AND THREE PLATES

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INTRODUCTION

The advance of mammalian cytology has been exceedingly slow, due partly to the difficulty of obtaining material showing the proper stages of mitosis in sufficient numbers but largely to the fact that the various methods of technique in use were not at all successful in preserving the structures of these small cells. The first difficulty is obviated by securing material from a large number of animals and examining a small portion of tissue from each animal (not more than one or two slides). Eventually a tissue may be found that is in, what might be called, a 'cycle of division,' when the mitotic figures are present in large numbers and a choice of good stages is readily made. This is true for germ as well as somatic tissue. The question of the preparation of mammalian tissue for cytological work forms the body of this paper.

During the early part of this work I had the benefit of the experience of Dr. Ezra Allen in his studies on the fixation of rat tissues (1) and Dr. McClung has assisted me all through my experiments with suggestions and criticisms. To these men I am exceedingly grateful.

Several years ago I began a study of the chromosomes of the pig but this had to be abandoned because of the hopelessness of analyzing the chromosome complexes. Even as my figures were then, they were as good as most of the published figures of mammalian mitosis. Since unlimited material was needed for experiments on technique and since there was a fairly large supply of cats available, the early work was carried out on cat testes and the method most successful on this tissue was tried on various other mammals. For some reason I have been unable to obtain the beautiful cytological preparations that Dr. Allen gets by the use of his picro-formol-acetic-chromic mixture, although I get excellent general fixation with it. This may possibly be due to the fact that his fixing fluid was developed with special reference to the rat. With the following method, however, I get very constant results which are, I believe, comparable in every respect to those obtained by Dr. Allen. Furthermore the fixation is not only constant for one mammal but has worked with uniformly good results on seven mammals and on the somatic as well as the germ cells. With two available methods in the field the difficulties besetting mammalian cytology should be much reduced.

THE FIXATIVES EXPERIMENTED WITH

In the experiments on technique that have been carried on in the Zoological Laboratory of the University of Pennsylvania for a number of years it has been found of value to vary the temperature at which fixing fluids are used. All the fluids tried by me were used as indicated at blood heat and at about 4 or 5°C. As I had previously failed to obtain good results at room temperature I did not, except in a few instances, experiment further at this degree.

An attempt was also made to study the effect of varying the time between the removal of the tissue from the body and the placing of it in the fixing medium. In the following table the

fluids used are outlined and also the methods of applying each fixative. In many cases the experiment was repeated when there seemed any chance of obtaining better results with that particular agent.

Table of fixatives

FLUID	TEMPERATURE	TIME IN FLUID	TIME THAT ELAPSED BEFORE FIXATION
	deg. C.	*hours*	
Bouin	38 and 4	24	Immediate
B.2 + urea (1)	38 and 4	21, 23, 24 and 49	Immediate and in 15 minutes
B.3 (2)	38	24	Immediate
B.3 + urea	38 and 4	24	Immediate
Carnoy	4	24 and 49	Immediate
Ohlmacher	4	24 and 48	Immediate and in 15 minutes
King's solution	4	4, 6, 21 and 25	Immediate
Allen's solution No. 15	38	24	Immediate
No. 16	38	24	Immediate
Flemming strong solution	Room, 38 and 4	24, 28, 30 and 49	Immediate
Flemming + urea strong solution	38 and 4	24 and 49	Immediate, 10, 15, 20, 25, 30, 45, 60 minutes and 4½ hours.

(1) and (2). B.2 and B.3 are the symbols which are used in this Laboratory to designate two modifications of the picro-formol-acetic preservative. These modificatons are:

Saturated	*B.2 parts*	*B.3 parts*
Picric acid, aqueous solution	75	75
Formalin	10	15
Glacial acetic acid	10	10

To these two solutions are sometimes added 0.5 gram of urea per hundred cubic centimeters.

In controlling the temperature of the fluids I have made use of our constant temperature rooms in the case of material fixed at 38°C. When the fluids were used cold the vials containing the fixatives were packed in ice in a quart 'Thermos' vacuum jar having a very wide mouth. As this jar is provided with a handle it is very convenient to carry about when it is necessary to go some distance for material. Flemming's solution when kept on ice registers about 4° to 5°C. Ice can be kept in this jar for several days.

The results of the experiments

The judgment that is passed on material prepared for cytological work must be based on two points:

1. *The general fixation.* If this is not good (i.e., if shrinkage or general distortion is evident) it paves the way for a very just criticism of any conclusions that may be drawn from the study of the chromosomes in such a tissue. As a rule if the general fixation is poor the chromosomes are not very likely to be decipherable, but should they appear so in shrunken and poorly fixed tissue they might well be regarded as probably abnormal.
2. The *clearness* of the various stages of the chromosomes and particularly, I think, of the *distinctness*, *differentiation* and *separation* of the chromosomes in the metaphase plate. In mammals, chromosomes that are more or less lumped together, even when it is apparently possible to distinguish the separate elements, do not give a true picture, in either form or number, of the actual conditions. This, I believe, I will be able to demonstrate a little later in this paper. Accepting material in such condition as workable has led several investigators of mammalian chromosomes rather far astray.

With these points in mind the material fixed by the above methods was studied. Most of the fluids used gave good preparations for general histological structure but the chromosomes were clumped and indistinguishable one from another. The first result obtained that approached the fulfillment of the required conditions was when cat testes was placed in cold (4° to 5°C.) Flemming's solution to which had been added a little urea. Previous to placing it in the fluid the tissue had been allowed to remain on a glass plate for approximately from ten to twenty minutes. Urea was added, as it had been found in the work of other members of the Laboratory to increase penetration. The amount taken has never been accurately determined but a few crystals are added to 5 or 10 cc. of fluid bringing the concentration of the urea in the fixative probably to about 0.5 per cent. It is difficult even to surmise why this method of delayed fixation should prove successful. It might be suggested that evaporation or exosmosis might play a part in separating the chromosomes although if this were true we would expect to find considerable shrinkage of the tissue. Slight shrinkage is to be observed in tissue treated in this manner although the chromosomes appear to be in excellent condition as is evidenced by figures 3, 5, 6, 7, 8, 9 and 11. This procedure gave excellent separation of the chromosomes in the testes and ovaries from a number of cats and with the testes of a few pigs. It was successful with the only cat embryo that was fixed in this way.

It was soon found that mammalian tissue could be fixed with better results, as far as lack of shrinkage was concerned, than those obtained by the above method if the material was cut into very small pieces (not more than 2 or 3 mm. in diameter) and placed immediately into cold Flemming plus urea. It is well not only to cut small pieces but to actually tease the tissue finely in the cold Flemming solution so as to obtain rapid penetration. When pieces too large are placed in the fixing solution good results are only obtained in a narrow area around the outside of the piece. The tissue may or may not blacken when placed in the cold Flemming. The absence of the usual blackness is not necessarily indicative of poor fixation.

WASHING AND DEHYDRATION

All Flemming fixed material was washed for about twenty-four hours in running water or, when this was impractical, many changes of water were made. Dr. Allen's automatic dehydrating device (1) is a great saver of time as it does away with the necessity of handling the material so many times. In the Laboratory I use it constantly but it is impossible to make use of it in field work. I have obtained as good results by dehydrating in the usual way making the steps in the ascending series of alcohols very gradual.

CLEARING AND EMBEDDING

When the material is in 95 per cent alcohol cedar oil is added and is usually allowed to remain on the tissue at least twelve hours, one or two changes being made during this time. The cedar oil is displaced with xylol and the material is embedded in paraffin in the usual way (2).

DISCUSSION OF THE SUCCESSFUL METHODS OF FIXATION

The latter method (i.e., involving the immediate placing of the tissue in the fluid) is not, as is the first, open to the possible criticism that the length of time between the removal of the tissue from the body and the placing of it in the fixing medium might allow pathological changes to enter. At present I am not of the opinion that the short wait between the removal and killing does produce anything abnormal other than a slight shrinkage. The chromosomes appear sharp and well separated and exhibit considerable differentiation. Some difference in the general morphology of the chromosomes is apparent in figures 10 and 11. Figure 10 is from the testes of a guinea-pig that was fixed immediately while figure 11 is from a testis of another individual fixed by the 'delayed' method ten minutes). The chromosomes in the 'delayed' fixation (fig. 11) are thinner and less differentiated than those of figure 10 although they are better separated. Whether this is a result of the method of preparation or not I cannot say at present. It will take considerable study and comparison to determine exactly whether this technique produces any abnormalities in the chromosomes and this study I have not completed as yet. However, since the plan of immediate fixation in cold Flemming plus urea has proved universally successful with mammalian tissue in my work, and as it is not open to the criticism pointed out above, I should suggest that it be followed in preference to the other method, using the delayed fixation only in case of the possible failure of immediate fixation.

Another point of great importance—no matter which method is followed—is that the material used must be absolutely fresh. This cannot be emphasized too strongly and is another condition that may help to account for some of the inaccuracies in the published results of mammalian chromosome studies. Early in my work on cat testes I found that testes removed from cats that had been dead not more than a half hour and whose bodies were still warm were hopeless for a chromosome study. Recently I have confirmed this observation on the testes of ten pigs which were received after having been removed from the animals for from ten to thirty minutes. The general fixation was excellent but the chromosomes were clumped together[1] and there was no recognizable differentiation in the size and shape of the chromosomes such as is discernible in tissue which has been killed when absolutely fresh. In text-figure 1 are shown the round shapeless masses of chromatin that result from improper fixation or from the fixation of stale tissue. Such figures do not give a true picture of the actual structure of the chromosomes and it seems very likely that frequently several chromosomes may be fused thus giving an erroneous impression of the total number. I recently prepared the testes, as they were removed, from eight pigs and the fixation (immediate immersion in cold Flemming) was excellent in each case. Figure 13 was drawn from a cell in this lot of material. Text figure 2 is a photograph of a brain cell in a well fixed pig embryo. Judging from the number of chromosomes that have been reported for the pig (eighteen) (3), my own early results (about twenty-four) on this animal in comparison with my present results (over forty) it would seem exceedingly probable that a fusion had taken place between many of the chromosomes in the studies showing lower numbers. This cannot be accounted for by assuming that a difference in the breed of pig might cause the great variation as I have, among others, material from the same breed as reported on by Wodsedalek (Poland China). Figure 3 represents a spermatogonial division in a Poland China boar. It might be suggested at this point that staleness of tissue as well as improper technique may account for the great variation in the number of chromosomes reported for human material as it is very difficult to obtain this tissue in an absolutely fresh condition. It might also be suggested in view of the fact that all the mammals studied by me have over forty chromosomes that when a perfect fixation of human material is attained the number of chromosomes will be

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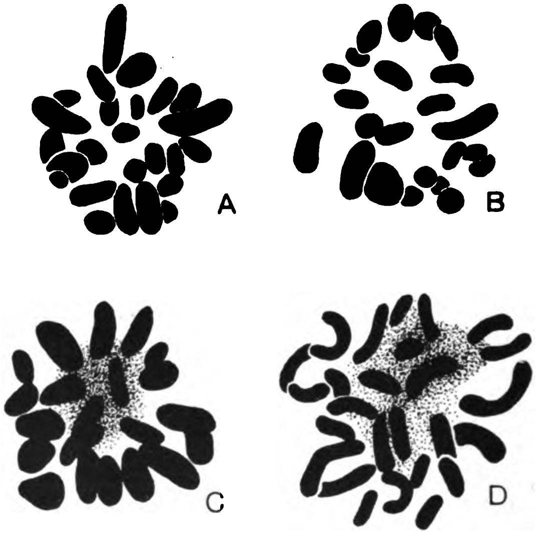
[1] The tendency for closely associated chromosomes to fuse and appear as one when poorly fixed has been strikingly illustrated in the studies of Dr. P. W. Whiting and myself on the chromosomes of the mosquito. In these studies the actual conditions, particularly in the somatic cells, were long obscured by the tendency for closely approximated chromosomes to run together. This condition has been discussed in our papers (4 and 3).

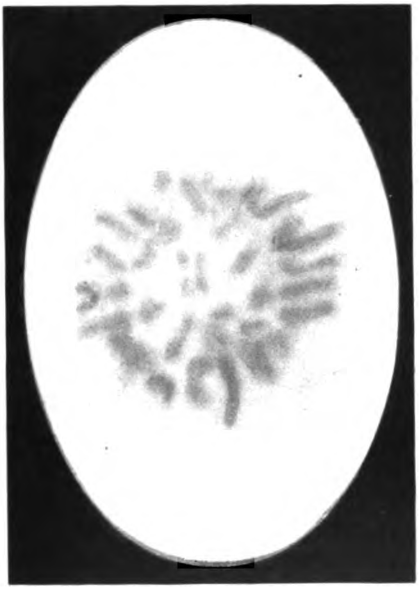


Text fig. 1 All four figures represent spermatogonia of the pig in metaphase. *A* and *B* illustrate the effect of poor fixation on fresh tissue. The unequal size of the chromosomes would seem to indicate that a fusion between various elements has taken place, and the massing together of these bodies in many cases make it impossible to distinguish the line of separation. Note that the chromosomes tend to be globular in form and lack differentiation.

C and *D* show the effect of the new method of fixation (immediate immersion in killing fluid) on stale tissue. *C* was taken from a testicle that had probably been removed from the animal for a longer period of time than had *D*. The large size and small number of the chromosomes of *C* again suggest that a fusion between chromosomes has taken place. The stipled area is dense and very indistinct. *D* shows a cell in a slightly better degree of preservation. The chromosomes are more numerous and better differentiated than in *C*. The form compares more favorably with the morphology of the chromosomes found in properly preserved material. In *D* there is also considerable density and lack of distinctness in the center of the chromosome plate indicated in the drawing by the stipled portion. Compare these drawings with figures 1, 2, 3, 4 and 13.

nearer that reported by Winiwarter (forty-eight) than that reported by other investigators.

The fact that a testis that remains in a dead body for a half hour or stands in a gross condition after removal from the body for a similar length of time or even less, shows the chromosomes clumped while tissue that is freshly removed, cut into small pieces and allowed to stand for ten to twenty minutes before immersion in the killing fluid presents the mitotic figures in apparently excellent condition seems to be contradictory. For the present the fact must stand without explanation as I can offer no suggestion as to the possible reason for this behavior.



Text fig. 2. This is an enlarged microphotograph of the metaphase chromosomes from the brain of a pig embryo. Figure 2 is a drawing of this cell.

It seems probable that the explanation for the success of the cold fixation may lie in the suppression of metabolic activities when the tissue is immersed in the cold medium and the preservation of the living structures until the fluid is able to penetrate and fix them permanently.

A notable feature of the immediate fixation of mammalian tissue in cold Flemming is the perfect preservation of the extremely delicate splits between chromosomes and of the chromosome forms found in the spermatocyte divisions. These figures have seldom been demonstrated in mammalian studies. Synizesis was not seen in this material except in the center of a piece of tissue which was rather large and where the fixative had not penetrated. Even here it is not the tight ball of threads figured by so many but gives every evidence that it is the result of the extraction of fluid. In my preparations where any evidence of the synizesis of the thin chromatin threads occurs, there is only a slight contraction of the threads away from the nuclear wall appearing as though the fluid which had supported these threads had been removed. The shrinkage usually appears to be equal from all sides although occasionally a cell is found with the chromatin threads massed at one side. In well teased or small pieces of tissue these same stages appear with the threads well separated and there is no shrinkage away from the nuclear wall. That there is something different at this stage is evident but the difference appears to me to be purely a physical one.

I have been much interested in the universality in application of the two methods of fixation of mammalian chromosomes outlined above, I have obtained excellent results with them on the following tissues in the number of cases indicated under the tissue in question. The number in parenthesis under the column

	TESTES	OVARY	EMBRYO	METHOD OF FIXATION
Cat	13	7	1	Delayed (5, 6, 7) and immediate (17)
Rat	4			Delayed and immediate (14 and 15)
Guinea-pig	2			Delayed (11) and immediate (10)
Pig	13		Several	Delayed (3) and immediate (1, 2, 4 and 13)
Rabbit	1			Delayed (8, 9)
Bat		2		Immediate (12)
Mouse	4			Immediate (16 and 18)

headed "Method of Fixation" refer to the figures illustrating the conditions found in the material. As used above 'delayed' indicates that the tissue has been allowed to stand in open after having been removed from the body (for from ten to twenty minutes) while 'immediate' indicates that the tissue had been cut into very fine pieces or teased and plunged immediately into the cold Flemming solution upon removal from the body.

In the case of the pig most of my material has been preserved by the immediate immersion in cold Flemming's solution plus urea. I have a large number of embryos fixed in this manner and as yet have examined only a few. Each one studied has shown uniformly good fixation so far and it may reasonably be expected that the remainder will be equally good since it is fairly evident that the results are not due to a happy accident in one or two isolated cases.

THE METHOD IN BRIEF

To fix mammalian material for cytological study:

1. Obtain fresh specimens from as many different animals as possible so as to be sure of obtaining one or more in a 'cycle of division.'
2. Place small or finely teased pieces of fresh tissue immediately into cold Flemming's solution plus urea. Allow to remain twenty to twenty-four or more hours.
3. It is suggested that this second method be not used except in case of the failure of the preceding one. Allow small pieces of fresh tissue to remain in the air for from ten to twenty minutes after removal from the animal before placing them in Flemming's solution plus a little urea which is used at a temperature of about 4°C. Allow the material to remain in the fluid for twenty to twenty-four or more hours.
4. Wash in water for about twenty-four hours.
5. Dehydrate by very gradual steps.
6. Clear from 95 per cent alcohol in cedar oil followed by xylol.
7. Imbed in paraffin.

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EXPLANATION OF PLATES

I have used drawings instead of photographs to illustrate the state of preservation in the various cells since drawings show the chromosomes much more satisfactorily than do photographs in most instances. Furthermore it is difficult to find cells in animals possessing so many chromosomes in which the metaphase plate is flat enough to photograph well although it may be perfectly easy to draw the same plate. I have included one photograph, which, though not good, is probably sufficiently clear to give an idea of the separation of the chromosomes and of their large number (text-fig. 2). Figure 2 is a drawing of the same cell.

All figures were drawn using a Zeiss apochromatic, 1.5 mm. objective and the compensating ocular 12 × which gives an original magnification of 3400 ×. These drawings were enlarged to twice the original magnification and reduced to approximately 4500 × in reproduction.

PLATE 1

EXPLANATION OF FIGURES

1 Prophase (late) from the amnion of a pig embryo. Immediate fixation.

2 Polar view of metaphase from the brain of a pig embryo. Immediate fixation.

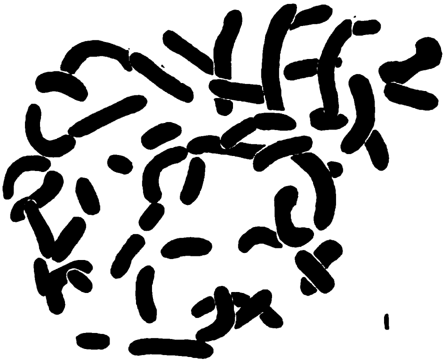
3 Polar view of metaphase of spermatogonium of pig. Delayed fixation.

4 Polar view of metaphase from the brain of a pig embryo. Immediate fixation.

5 and 6 Polar views of metaphase of spermatogonia of cat. Delayed fixation.

FIXATION OF MAMMALIAN CHROMOSOMES
ROBERT J. HANCE

PLATE 1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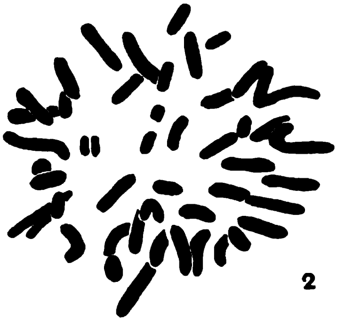
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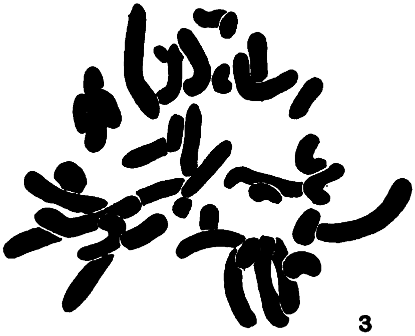
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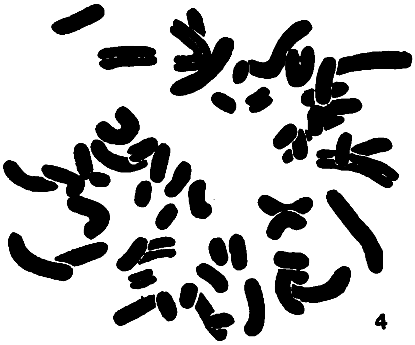
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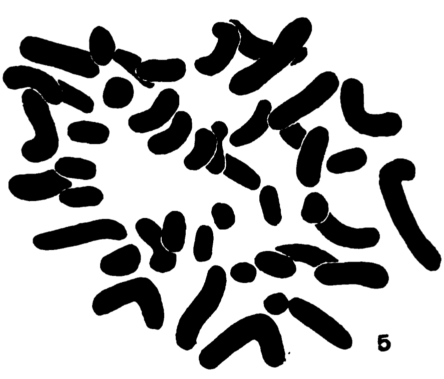
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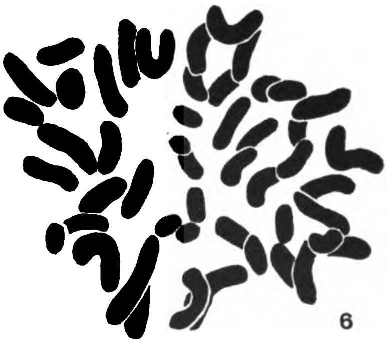
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PLATE 2

EXPLANATION OF FIGURES

7 a and b Polar view of spermatogonium of a cat found in two sections. Delayed fixation.

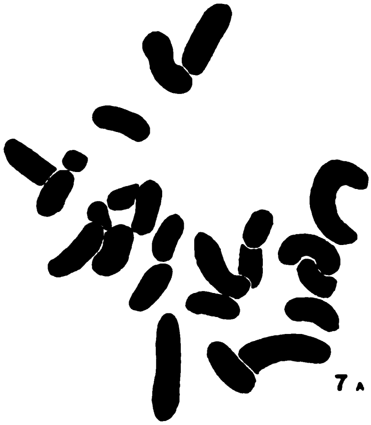
8 Side view of the metaphase plate in the spermatogonium of a rabbit. Delayed fixation.

9 Prophase of spermatogonium of rabbit. Delayed fixation.

10 Polar view of metaphase of spermatogonium of a guinea-pig. Immediate fixation.

11 Polar view of metaphase of the spermatogonium of a guinea pig. Delayed fixation. Note the difference in the chromosomes figures 10 and 11.

12 Polar view of metaphase in the ovary of a bat. Immediate fixation.



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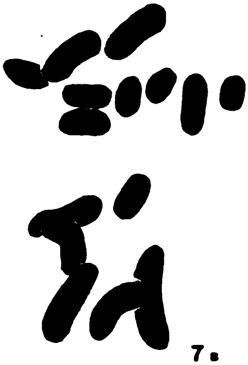

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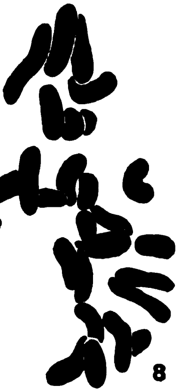

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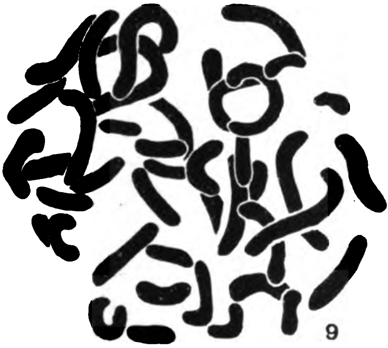

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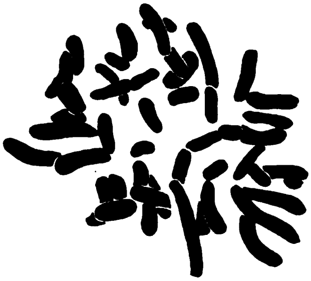

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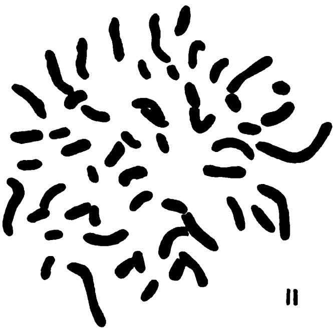

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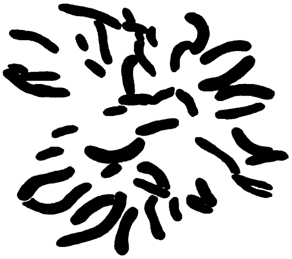

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PLATE 3

EXPLANATION OF FIGURES

13 Polar view of metaphase of spermatogonium of pig. Immediate fixation.

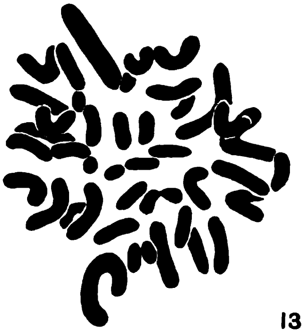
14 Late prophase of spermatogonium of rat. Immediate fixation. This is probably a cut cell.

15 Metaphase of spermatogonium of rat. Immediate fixation. This is a cut cell.

16 Early prophase figures from a first spermatocyte of a mouse. This is only a portion of the cell. Immediate fixation.

17 First spermatocyte figures from the testes of a cat. Note the characteristic forms that have been reported for other forms but seldom for mammals. Immediate fixation. Such forms have been found in all the mammalian tissue studied.

18 A first spermatocyte from the testes of a mouse. An oblique view. Immediate fixation. This is probably a cut cell.



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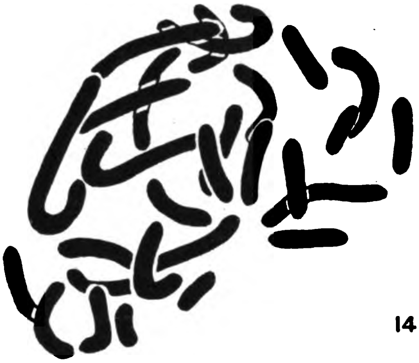

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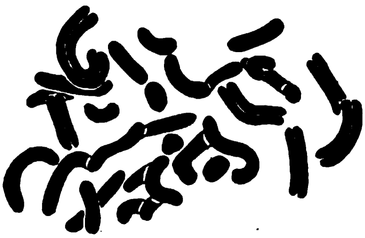

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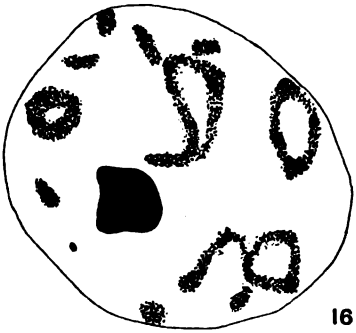

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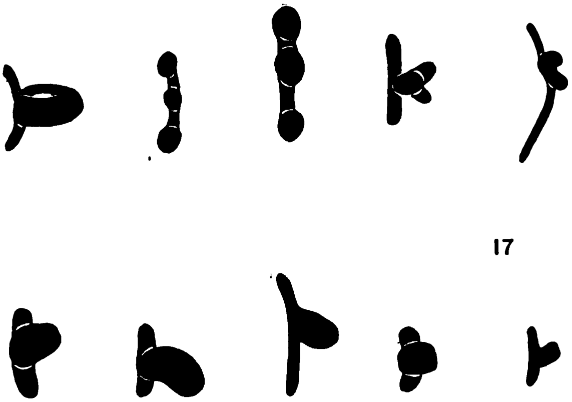

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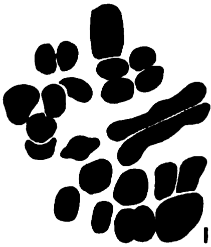

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STUDIES ON A RACE OF PARAMOECIUM POSSESSING EXTRA CONTRACTILE VACUOLES

I. AN ACCOUNT OF THE MORPHOLOGY, PHYSIOLOGY, GENETICS AND CYTOLOGY OF THIS NEW RACE

ROBERT T. HANCE
Zoölogical Laboratory, University of Pennsylvania

THREE PLATES AND TWELVE CHARTS

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I. INTRODUCTION

While examining a number of paramoecia taken from a laboratory culture in the early part of January, 1915, one individual was seen to have three instead of the usual two contractile vacuoles. Subsequent examination of the stock from the original culture showed that a large percentage of the animals possessed the extra organs. Several animals with three vacuoles were therefore isolated and pure lines started from them. It soon became evident that the potentiality for one supernumerary vacuole was not only inherited but that the offspring might possess even more than one extra contractile organ. A preliminary account of the behavior of these organs in heredity was published some months ago (2).

The discovery of such a marked variety of a form which has served as a basis for so much valuable study opens an interesting field for investigation. Parts of this report must necessarily be of the most preliminary sort and various portions will be completed from time to time and published separately. The finding of the new race of paramoecium affords opportunity for comparison with the common race and for a study of the physiological activities of the two varieties. The following points will be considered more or less completely in the following paper:

1. The extra vacuole gives a definite character in the Protozoa whose inheritance can be traced. The existence of two races differing as far as is known only in this one character gives an opportunity of crossing the two races by conjugation and of determining whether inheritance in protozoa is Mendelian as it is in the metazoans.
2. A comparison of the two races may be helpful in the analysis of the function of the contractile vacuole.
3. Some light may be thrown on the process of conjugation through a study of the behavior of both races.

II. TECHNIQUE

A separate pipette was used for each culture to guard against cross contamination and was hung in a special holder attached to the jar when not in use (3). In following the offspring of a single individual for several generations each of the daughter cells was isolated in a watch glass and as fast as the individuals divided a record was made of the number of vacuoles in each and they were again separated. The syracuse watch glasses that were used were sterilized in boiling water to which a little clean paraffin had been added. This deposited an invisible coating on the glass which was, however, sufficient to prevent the drop of hay infusion from spreading over the surface as it would on perfectly clean glass. On the paraffined surface the fluid rounds up and presents the minimum surface for evaporation (3). It was found that the most satisfactory pipette for this work had a short tip of almost hairlike fineness. The method of making these has been described (3).

It is rather difficult to find a method of slowing down the animals for the purpose of making accurate observations without killing them. The first method used was to place an individual in a small drop of water and over this a cover glass was lowered with the aid of a fine pair of forceps. The cover had a drop of hardened balsam at two corners which had been filed down to the proper thickness and which prevented it from crushing the animal. The excess fluid was drawn off with a piece of filter paper. To recover the animal the slide was tilted over a watch glass and by means of a stream of hay infusion the cover and whatever was under it were flushed into the crystal. This method was very laborious and slow, and the chances of losing the paramoecium great. The following method has proved to be the better: An individual is placed on a slide in a fairly large drop of the medium. The liquid is drawn off with a pipette under the dissecting microscope, the animal being kept as nearly as possible in the center of the drop. Finally, enough of the fluid is removed so that the adhesion of the surface film to the slide exerts sufficient pull to hold the animal quiet. In the center of the drop the pressure is least and it seldom causes a paramoecium to burst. Toward the edges, however, the chances are very great that the animal will succumb almost instantly to the greater pressure. In such a preparation the pressure at the center tends automatically to lessen as the surface tension slowly draws the liquid from its close adhesion to the slide and rounds it up. This takes a sufficient length of time, however, to allow accurate observations to be made under the compound microscope. Occasionally the pressure is relieved too quickly and the paramoecium is given enough fluid to move about in, which makes observation with the high power impossible and the processs has to be repeated. As soon as the examination has been made, a drop of fresh hay infusion is added. To drop this liquid immediately on top of the animal after it has been under pressure may frequently crush it and the safer way is to allow the fresh fluid to flow from the side into the drop containing the animal. The paramoecium may then be picked up in a pipette and placed in its respective watch glass. When examining paramoecia in pure lines a separate pipette is used for each line.

Under the 16 mm. lens when the animal is compressed, the vacuoles stand out with diagrammatic clearness appearing as so many holes punched through the cell and as a rule are to be found close to one side and almost invariably lying in a straight line. This latter characteristic was one of the criteria for differentiating between the contractile vacuoles and food vacuoles when these were so numerous as partly to obscure the former. The comparative lack of refraction of the pulsating organs was another basis of distinction. When, however, the refraction of the food vacuoles was almost identical with that of the contractile vacuoles under the low power, the 4 mm. lens was swung into place and the contraction of the supposed contractile vacuoles was watched for. It has been observed that the vacuoles in animals raised in watch glasses are more difficult to see than they are in animals living in larger amounts of medium. In the watch glasses the food vacuoles have a lack of refraction which gives them an appearance very much like the contractile vacuoles whereas the food vacuoles in animals raised in the large culture jars frequently appear very nearly black.

Cytological technique. For measuring and drawing the whole paramoecium Worcester's fluid was used (10 per cent formalin saturated with mercuric chloride). Animals fixed in this way can be immediately cleared in glycerine which brings out many of the internal parts very well, and the vacuoles, if they happen to be expanded at the time of fixation, are visible. For whole mounts and sectioning, paramoecia were fixed in Schaudinn's, Gilson's, Flemming's and Worcester's solutions. As comparatively little time has been devoted to cytological studies I am not prepared at present to offer any criticism of the fixatives.

To Dr. M. H. Jacobs I am indebted for his constant interest, advice and criticism during the progress of the work recorded here and I also feel under great obligation to the other members of the Zoological Department of the University of Pennsylvania whose many kind suggestions have been of great assistance to me.

III. MORPHOLOGY

a. General description

In general form, the animals of this race are apparently identical with the common slipper-shaped Paramoecium caudatum. The newly discovered individuals average rather larger than any of the representatives of the two-vacuoled race I have found about Philadelphia. Chart 1 shows a curve plotted to illustrate the range in size. The peak of the curve is at 233 μ and is very nearly midway between the two extremes. On this same chart are plotted the ends of two curves formed by animals taken about Philadelphia. The range of one race is from 153 μ to 207 μ and in the other from 106 μ to 173 μ. In the collection of slides belonging to the Department I have found paramoecia somewhat larger than the new race ranging from 197 μ to 325 μ. The extremes of all the races studied by Jennings were 50 μ and 332 μ.

In certain cultures where the individuals were unusually favorable for study a band slightly darker than the surrounding protoplasm could be seen across the center of the animal in the region where the constriction appears at the time of division (figs. 4 and 6). It is interesting to note that in these cultures

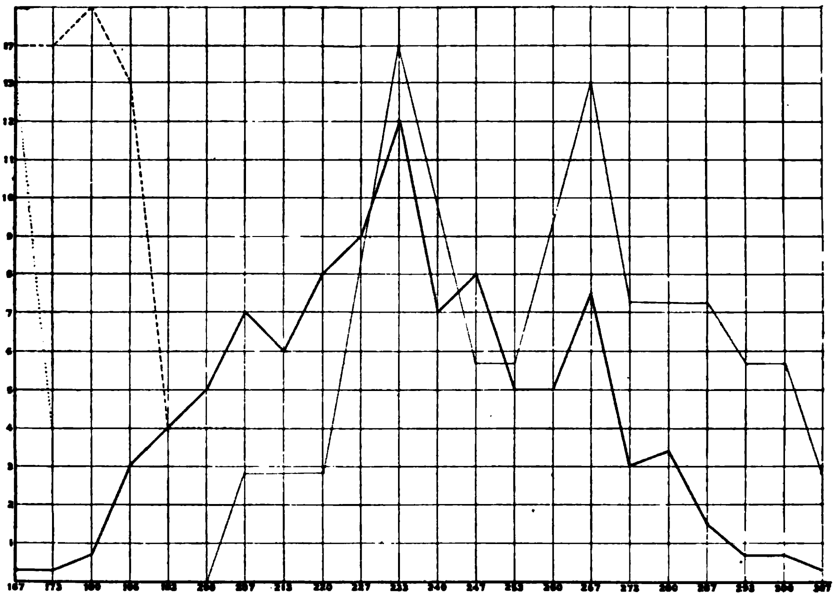


Chart 1 On the axis of abscissas are marked the various sizes in μ while on the axis of ordinates are placed the percentages. The heavy solid line represents the multivacuoled race of paramoecium while the light broken line indicates the larger sizes of a small two-vacuoled race and the light unbroken line is based on figures obtained from fixed preparations of two-vacuoled animals in the Laboratory collection.

the percentage of animals with three and four vacuoles was very high. The consideration of this phenomenon will be left for future work.

b. The vacuoles

1. Location. So exactly are the contractile vacuoles located that a single straight line parallel to the outline of the animal might be drawn passing through the center of each. In a very few cases I have found one or two vacuoles slightly displaced from the usual straight row condition (fig. 9). In the majority of individuals the extra vacuoles are located in the posterior part of the animals as can be seen from the drawings. In a few instances, however, one extra vacuole was found in the anterior end (fig. 3), but I have never found more than two vacuoles located in the foremost half of the body. It is a curious fact that, rare as this condition is in the normal medium of hay infusion, in cultures to which a little sea salt has been added paramoecia with two vacuoles in the anterior end have been found to be much more numerous. As yet no plausible explanation for the increase of this rare arrangement has been found—if, indeed, it is not merely a coincidence.

There can be no doubt that the contractile vacuoles are very definitely located organs and fixed in position for a given individual. When an animal is swimming the vacuoles can be seen to turn with the cell and always maintain the same position in relation to each other. As far as I have been able to determine, both the radiating canals and what appears to be the excurrent pore or tube aid in holding the vacuoles in position and particularly the latter. That there is a connection with the outer wall of the animal can be seen when a paramoecium bursts directly opposite to a fully expanded vacuole, when the vacuole, drawn by the outflowing protoplasm, can be seen straining at some retaining fastening.

2. The vacuoles. Under normal conditions the vacuoles in this race measure on the average about 10 μ in diameter with a capacity of 500 cubic micra. There may be some variation in this size due to the age of the vacuole, i.e., the length of time since it had appeared, but I think that it is safe to say that the diameter of fully formed vacuoles is very nearly the same. There can be no question that these vacuoles originate separately as they generally lie at some distance from each other and no cases have been seen when a vacuole appeared as though it had been separated by division from another vacuole. The average size of the vacuoles in the common race which I have observed is 11 μ with a cubic content of 664 cubic micra. In my fixed preparation the vacuoles are surrounded by what appears to be a definite morphological membrane (fig. 16). When animals have been kept at a temperature a few degrees above freezing for a few hours the vacuoles expand and do not contract and it is interesting to note that the anterior vacuole becomes from three to four times the diameter of those in the posterior end. This, in the cases studied, was not true for the common two-vacuoled race, neither vacuole, exceeding the other in size.

IV. OBSERVATIONS ON THE BEHAVIOR OF THE VACUOLES

To sum up briefly our knowledge of contractile vacuoles:

The contractile vacuole is a pulsating organ surrounded by a membrane of physiological importance, belonging to the ectoplasm. These vacuoles form an osmotic system and the evidence at hand shows them to function in part as excretory and respiratory organs.

a. Percentage of variation in contractile vacuole number

After the pure lines that had been started had multiplied considerably examination was made of a number of individuals in each line. Apparently these high numbers of vacuoles were not in every case passed on to the offspring for in all of the cultures, whether started with three or four vacuoled paramoecia, there were considerable numbers of two vacuoled forms. But on the contrary in the pure lines started with three vacuoled forms there were found individuals possessing four contractile organs. Observations made on one culture in the early days of the work showed a range of variation in vacuole number in the following percentages.

	per cent
Two vacuoles	8.6
Three vacuoles	65.7
Four vacuoles	25.7

Later and more accurate observations were made for a period of thirty days on one culture (covering 663 individuals) and the results for that time averaged showed the proportion to be:

	per cent
Two vacuoles	16.13
Three vacuoles	66.48
Four vacuoles	16.43
Five vacuoles	0.904

In both of these tables, and in all of the following work, the three vacuoled animals show themselves to be in the excess in nearly all cases. The statement made in my first note on this race that there was an apparent tendency for the vacuole number to settle down with three as the standard must be retracted. When the note was written I was not familiar with the response of the vacuole number to various conditions (rate of division, age of medium, etc.).

The last table shows that a five-vacuoled paramoecium appeared. Animals with five vacuoles, while not rare, seldom have been found in a percentage greater than the one indicated. The cause of the limited production of these extreme forms will be discussed under Section VI. Recently four paramoecia have been obtained with six contractile vacuoles and one with seven (figs. 10 and 12). It is a striking sight to see a paramoecium with six or seven vacuoles in a row all swelling and contracting regularly. The six vacuoled condition is very rare and in over a year I have not seen more than a half dozen animals thus equipped, and the majority of these I have found recently. The only cases seen in 1915 were very doubtful as the contractile vacuoles were hidden by food vacuoles of similar refractiveness.

The above results were obtained on cultures maintained under relatively similar conditions and it is interesting to compare them with the average of the results obtained from a number of cultures under observation for a much longer time and under much more varied conditions. The data from which charts 2, 3, 4 and 5 were made served as a basis for these calculations. These data were obtained on paramoecia under exceedingly varied conditions—in cultures ranging from freshly made up infusions to cultures that were very old, in cultures where plenty of medium was present and in cultures that were undergoing rapid evaporation, in culture during conjugation and after the process had stopped. The averages given below are based on an examination of 9818 animals.

	per cent
Two vacuoles	23.5
Three vacuoles	61.3
Four vacuoles	13.4
Five vacuoles	0.22
Six vacuoles	0.042
Seven vacuoles	0.0001

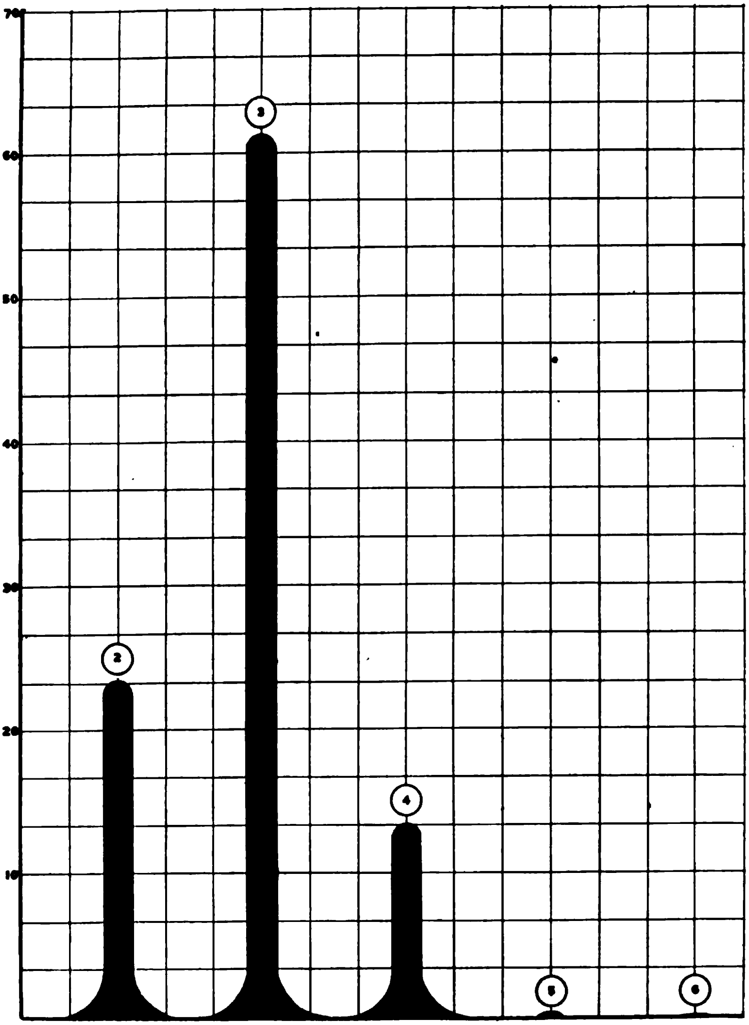
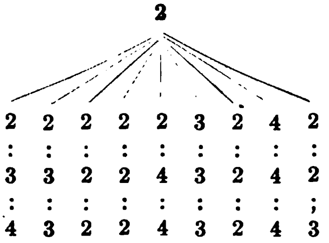


Chart 2 This chart gives a graphic illustration of the comparative numbers of two, three, four, five and six-vacuoled animals. This chart was prepared from data on ten thousand animals living under all conditions of medium.

Chart 2 illustrates these percentages graphically. In comparison with the twos, threes and fours, the fives and sixes, and especially the latter, scarcely appear above the base line.

b. Variability in vacuole number in the life cycle of the individual

While making the above studies it became evident that the time of day apparently had some influence on the various percentages. This suggested that the period in the life cycle of the individual at which the observation was made might play a rôle in determining the general percentage of the various vacuole numbers. (In this paper reference to the life cycle of the paramoecium always refers to the period between transverse divisions.) A single individual showing two vacuoles was therefore isolated and allowed to divide several times. Examination was then made of all the offspring and each was isolated in a separate watch glass. At intervals of from three to five hours the animals were again examined with the following results.



As can be seen, paramoecia possessing two contractile vacuoles increased this number in several cases before division. In some of the individuals the number was not increased. However, continued work with the two vacuoled forms has demonstrated that they have not lost their power of producing multi-vacuoled daughters. In one case I have followed individuals through three generations (representing fifteen animals in all) without a three vacuoled form appearing and then the extra vacuoled animals were produced in just a large percentages as in other lines. This point will be more concretely illustrated under 'selection,' page 316.

The potentiality for the higher number of contractile vacuoles is maintained although several generations may be passed before it exerts itself or, better, before the conditions are such that it must exert itself.

c. The number of contractile vacuoles in the offspring

When normal individuals get ready to divide, and when the beginning of the constriction has appeared, two new vacuoles are added so that immediately after division the daughter cells are fully equipped with excretory organs. In this new race, as a rule, two vacuoles are added at this time, although cases appear when only one seems to be formed. In a certain percentage, apparently more than two have been added before division but whether the vacuoles in excess of two were added before or after division cannot be ascertained from my data, as the observations were not, as a rule, made until some time after separation had taken place, in which case the extra vacuoles might have been formed after division. In a few cases division occurred without the formation of any new vacuoles.

For 225 animals in:

15.11 per cent—one vacuole was added
54.66 per cent—two vacuoles were added
30.22 per cent—more than two vacuoles were added

From this it is evident that in at least 50 per cent of the cases the usual normal addition of two new vacuoles prevails as in the common race.

With these facts in hand, the number of vacuoles possessed by the offspring of the individuals of this new race may be understood. It is practically impossible, however, to predict with certainty what number of contractile vacuoles the offspring of any particular paramoecium will have immediately following division. Some of the possibilities observed up to date are illustrated below.

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)	(n)
2	2	2	3	3	3	3	4	4	4	5	5	5	6
∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧
2 2	2 3	3 3	2 2	2 3	3 3	4 2	2 3	4 2	3 3	4 2	3 3	5 2	4 2

As stated above, as a rule two vacuoles are added at the time of division (one to the anterior end and one to the posterior end). a, e, i, j, and m represent cases where presumably such an addition has taken place. In b, c, f and g more than two vacuoles have been added although as pointed out above this may be due to post divisional additions rather than to additions at the time of constriction. d, h, and l represent cases where only one vacuole, has been added, while n indicates that there has been no increase whatever.

d. Effect of various conditions on the vacuole number

Division rate. Early in the course of these observations it appeared that in newly started cultures of fresh hay infusion where the animals were increasing rapidly the percentage of two-vacuoled forms was frequently very high. On the other hand, in older cultures where the division rate was presumably slower the percentage of multi-vacuoled animals outnumbered the twos. To test this point more accurately, a number of cultures were set up to see whether this condition was the rule. From the time the cultures were well established records were made of the percentage of individuals possessing two, three, four or five vacuoles in each jar every day, or every few days, extending over a period of from two to three months. These records supplied the data for drawing up the accompanying graphs. The results of these experiments have been plotted in various ways to show conclusively that the mode of behavior is essentially always the same.

In all the charts the three-vacuoled animals are represented by a heavy broken line, the twos by a heavy unbroken line and the fours by a light unbroken line and when the fives are drawn in a light broken line is used. The figures placed across the various curves in all the charts except No. 5 indicate the number of vacuoles in the form which the curve represents. Chart 3 represents a single culture It can be seen at a glance that although the twos are at first more numerous their line drops rapidly until it meets the axis of abscissas on March 10 while the threes and fours rise to a certain point which is maintained with more or less fluctuation to the end. Chart 4 is drawn from figures based on the average of three cultures and shows the various curves to follow practically the same paths that they did in the preceding graph, and although the twos in this case

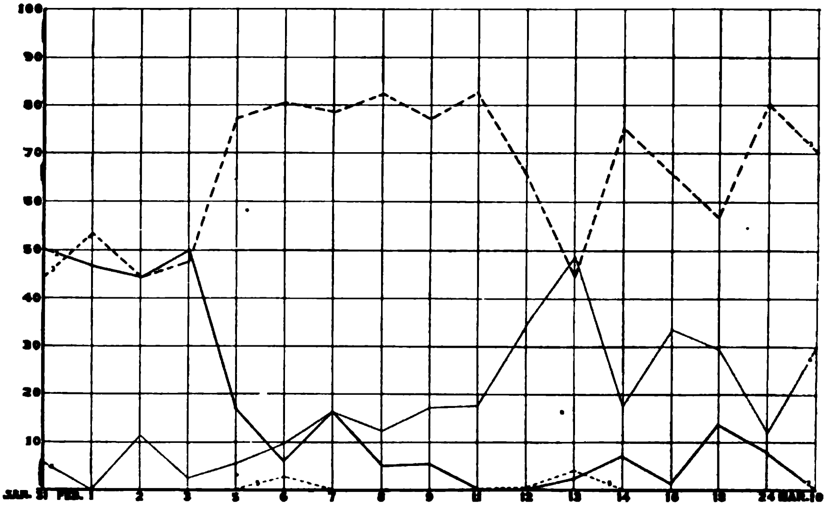


Chart 3 In this chart and in all succeeding ones the axis of abscissa represents time in days and the axis of ordinates represents percentages. The description of this chart is in the text, page 299.

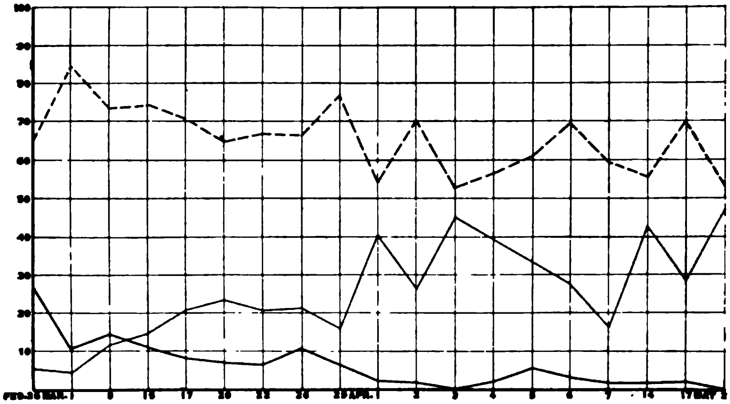


Chart 4 This graph is described on page 300.

did not begin in the majority, they nevertheless drop toward the base line noticeably. Chart 5 is a striking confirmation of the two previous charts. Six cultures were followed and the results plotted on the one chart. The fours play a comparatively unimportant part and will not be discussed. The X formed by the crossing of the unbroken line representing the two vacuoled animals falling from high on the axis of ordinates to the axis of abscissas by the dotted curve of the three-vacuoled paramoecia rising from low percentages to higher ones is very marked.

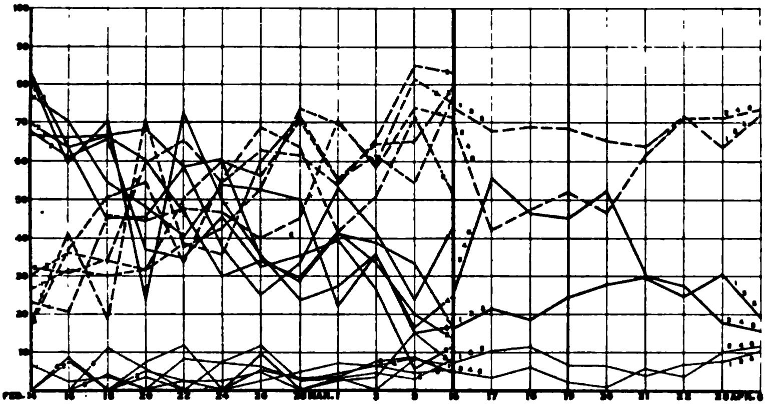


Chart 5 The description of this chart is on page 301.
The numbers on the various curves indicate the number of the culture and not the number of the vacuoles as on the other charts. The same method of designating the various forms is used as on the other graphs.

Still more interesting are the results obtained from March 15 on and represented on this graph. Cultures 1, 3 and 5 were undisturbed while fresh hay infusion was added to cultures 2, 4 and 6. The results for each three cultures were averaged and plotted. 1, 3 and 5 continued as before while 2, 4 and 6 due to the acceleration of the division rate caused by the addition of fresh infusion show a rapid increase of two-vacuoled paramoecia with a corresponding drop in the threes. By April 6, however, the rate of increase had slowed down and the curves of the one set of cultures were very close to the corresponding curves of the other set. Chart 12 while drawn primarily to illustrate the effect of conjugation shows conditions similar to the above. Chart 6 illustrates graphically the relation between the division rate and the percentages of animals possessing various numbers of vacuoles. The very heavy line represents the division rate and it can be readily seen how the percentage of two-vacuoled animals rises and falls with the increase or decrease of the fission rate.

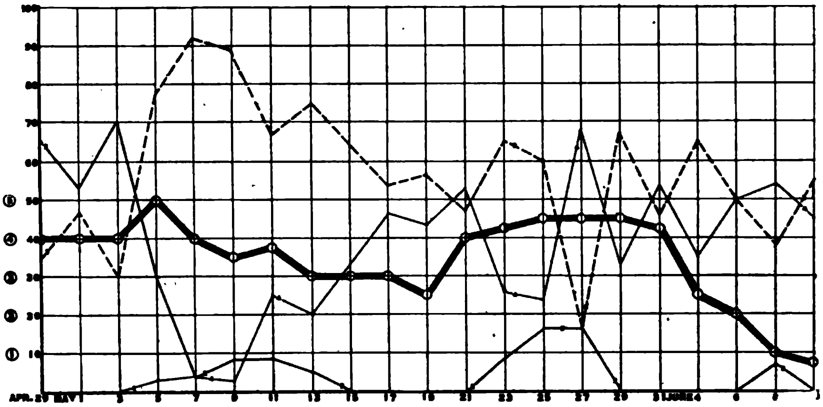


Chart 6 This graph compares the fluctuations of the number of two, three and four-vacuoled paramoecia in a single culture for a period of nearly two months with the daily division rate. The division rate was obtained by isolating a number of single animals in separate watch glasses in fluid filtered from the main culture. At stated intervals an average of the number of divisions that had occurred in all the watch glasses was struck. The figures in circles at the extreme left of the chart indicate the number of divisions and the broad black band traces the division rate across the graph. The other figures are the same as have been used on previous charts. It is evident from this chart that in general while the threes and fours increase as the division rate decreases there is apparently another factor at work that cannot be accounted for on this basis.

Age of the culture. To determine whether paramoecia divide more rapidly in fresh hay infusion than in an old, worn out medium, liquid was filtered from a culture nine months old. A few drops of this fluid was placed in several watch glasses and a paramoecium taken from the same culture as the liquid was isolated in each. From this same old culture single paramoecia were placed in similar amount of fresh hay infusion. In six days all of the animals isolated in the fresh medium had divided twice producing four animals, while one of the animals in the old culture had divided once, one had not divided at all, and two had died. Even in the new medium the division rate was apparently very slow, but the animals were so starved that it took them several days to return to the normal condition. After starved paramoecia have been abundantly fed I have always found their division rate to increase to normal.

This experiment was repeated using animals that were not starved. The division rate for a period of five days was 2.7 in the old infusion against 5.6 divisions of sister paramoecia in freshly made hay medium. A more complete account of the effect of various media will be given under Physiology.

V. PHYSIOLOGY

a. General

It has been over two years since the original animals of the new race were discovered and they not only show no indication of dying off but have given much evidence of being a line superior in vitality to any of the two-vacuoled paramoecia with which they have been compared. Cultures of this new race have been kept in various laboratories of the Zoological Department of the University of Pennsylvania and in the course of time, since no particular care was taken to prevent the mixture of various cultures, the multi-vacuoled animals found their way into the general cultures and it was not long before they had displaced the common form. For some time this year it was impossible to find a normal two-vacuoled paramoecium in any of the cultures maintained in this laboratory. This extra vacuoled race is made up of rather large animals and under many adverse conditions these paramoecia surpass the common races in viability and vigor.

One of the characteristics of the race in question that was first observed was the extreme resistance it seemed to possess to culture media which were so old that they would probably not have supported the common race of caudatum. Several cultures made up at the beginning of the summer recess came through the hot weather in a very healthy condition although the cultures had remained in a room and in which the temperature was at times quite high. Cultures are being carried along at present that are nine months old and although most of the animals have died there are still a few very active paramoecia remaining.

The division rate for this race has a somewhat wider range than I have observed in the case of other races. Under normal conditions (fresh or nearly fresh hay infusion) these animals divide from one to three times in twenty-four hours. When the culture medium is older the rate may drop below this. There have been times, however, when the animals under observations divided as rapidly as five times in twenty-four hours and this rate was maintained for six or seven days. This rate of division is much more rapid than is usual for the two-vacuoled races.

In an attempt to determine whether or not the rapidity of division is the sole agent in the production or inhibition of the formation of extra vacuoles, several single individuals were isolated in watch glasses containing hay infusions that differed widely in age and condition. Three sets of experiments were set up in each kind of infusion and in three days the number of divisions that had occurred and the percentage of two, three and four-vacuoled animals that were present was recorded for each of the three sets of experiments and for each type of medium. The results obtained in each infusion were averaged and are given below. All the experiments were repeated to determine whether the results of the first set would be constant. The cultures from which the animals for this experiment were drawn was started from a single individual. The number of divisions given is for a period of three days.

1. Medium filtered from culture nine months old

NUMBER OF DIVISIONS	EXPERIMENT A	NUMBER OF DIVISIONS	EXPERIMENT B
3	Two vacuoles, 44 per cent Three vacuoles, 48 per cent Four vacuoles, 8 per cent	2.7	Two vacuoles, 31 per cent Three vacuoles, 61 per cent Four vacuoles, 8 per cent

2. Medium filtered from culture from which paramoecia were obtained to conduct these experiments. Culture was about one week old

NUMBER OF DIVISIONS	EXPERIMENT A	NUMBER OF DIVISIONS	EXPERIMENT B
4.3	Two vacuoles, 81 per cent Three vacuoles, 18 per cent Four vacuoles, 1 per cent	3.7	Two vacuoles, 30 per cent Three vacuoles, 70 per cent

3. Hay infusion freshly made up

NUMBER OF DIVISIONS	EXPERIMENT B
5.6	Two vacuoles, 82 per cent Three vacuoles, 18 per cent

4. Medium filtered from culture containing many animals which had been allowed to evaporate (concentrate) and had been refilled with fresh hay infusion four times. The concentration of the metabolic products present was doubtless quite high and the culture had a very strong odor. At the time this experiment was started fresh infusion had been added to the culture about twenty-four hours

NUMBER OF DIVISIONS	EXPERIMENT B
4.5	Two vacuoles, 37.5 per cent Three vacuoles, 50 per cent Four vacuoles, 12.5 per cent

The foregoing data point strongly, I think, to the fact that rapidity of division plays a very important rôle in influencing the production of the extra vacuoles. Apparently one of the chief requirements for the production of the extra vacuoles for which this race possesses the potentiality is sufficient length of time between divisions. When the rate of division is rapid the two-vacuoled animals tend to predominate as illustrated by Experiment A, 2; and Experiment B, 3. With a slower rate of multiplication there appear more three and four-vacuoled paramoecia as the results of Experiments A, 1 and B, 1 and 2 show. Moreover, in old cultures where the division rate is low two-vacuoled animals are very scarce when not absolutely lacking. While there is some slight variation in the results of the corresponding sets of experiments I am not at present inclined to believe that this fact interfered with any of the above statements, as the variability of individuals may readily account for this. In general all of the results indicate that extra vacuoles appear as a rule when the rate of fission is relatively slow. Chart 6 compares graphically the division rate with the percentages of two, three and four-vacuoled, animals.

There are, however, enough exceptions to the general rule to suggest the probability of some other factors playing a part and very likely, not an unimportant part in determining the number of vacuoles. As yet, I am not certain what this factor or these factors may be, but Experiment B, 4 gives at least the basis of a working hypothesis. In this particular experiment although the division rate was high the number of two-vacuoled paramoecia developed was 43.5 per cent under the number produced in Experiment A, 2 where the rapidity of division was approximately the same. The difference between the experiments lay in that the fluid used in Experiment B, 4 contained many times the amount of metabolic waste products present in Experiment A, 2. From this result the tentative suggestion may be made that although the rate of division seems to be the primary agent in influencing the vacuole number, the presence of waste products in the environment has an activating effect on the production of extra vacuoles. Indeed if the amount of the waste products of metabolism is sufficient it may in part offset the effect of a rapid division rate.

Merely slowing down the rate of division is not sufficient, as this was tried by placing six paramoecia in a constant temperature room at 12°C. In four days two had divided only once and the rest not at all while the controls at room temperature had each divided three times. When the animals in the cold room were examined it was found that the vacuole number had not increased at all. Under these conditions although division did not occur for four days, the temperature lowered the metabolic activities of the paramoecia and made the production of extra vacuoles either impossible or unnecessary.

b. Resistance

This race has proved itself to be more resistant to all the abnormal conditions it has been subjected to than have the common animals.

Heat. Dr. Jacobs has found that under conditions where the ordinary paramoecia are killed at temperatures of 40° to 42° C. the race in question may survive an exposure to 44°. Animals that have been living in a hay infusion to which a small percentage of sea salt has been added resist high temperatures for very much longer periods of time than are paramoecia taken from pure hay infusion. My experiments along this line are, as yet, not sufficiently extensive to base conclusions.

Cold. A few animals of the multi-vacuoled race withstood an air temperature of

1° to 2° for 45 hours
−3° to −4° for 2½ hours

The common race was killed in the first case between five and thirty hours and in a trifle over an hour in the second experiment. In this time, although ice crystals were formed, the fluid did not freeze solid at this low temperature.

Distilled water. Mr. Mitchell Carrol, of the Zoological Laboratory of the University of Pennsylvania, has maintained this race of paramoecium in distilled water for fifteen generations, covering a period of forty-four days. In similar experiments of my own these animals showed the same ability to live in distilled water.

c. Rate of vacuole contraction

A few observations on the rate of vacuole contraction under varying conditions have been made on this new race of Paramoecium to determine whether there is any essential difference between this and the common stock. Two methods have been employed. In the first method each vacuole as it contracted was called off to an assistant who noted the time. Several observations were made on each vacuole and then the records obtained for each vacuole were averaged. In all forty-four animals were studied at 23°C. with an average of three observations per vacuole per animal.

	FIRST VACUOLE	SECOND VACUOLE	THIRD VACUOLE	FOURTH VACUOLE
Two vacuoled animals	9	9		
Three vacuoled animals	10	13	13	
Four vacuoled animals	11	12	14	9

The second method employed, though not so accurate as the first since only one observation per vacuole per animal was obtained, gave results which are approximately the same as the first. The time of contraction was taken with a stop watch from the instant the vacuole disappeared until it disappeared again. With animals possessing three or more vacuoles two watches were frequently used as the paramoecium would often dry up if each contraction was timed separately. The results obtained at each temperature are given below. The figures are based on observations of thirty-five paramoecia.

The well known fact that the rapidity of vacuole contraction increases with the temperature is again brought out in these tables. It is evident in the multi-vacuoled race that there is a

Multi-vacuoled race. Time in seconds; temperature in Centigrade

	FIRST VACUOLE	SECOND VACUOLE	THIRD VACUOLE	FOURTH VACUOLE	TEMPERATURE
					deg. C.
Two vacuoled animals	8 9 10	13 10 9			22.0 23.0 24.0
Average	9	11			
Three vacuoled animals	13 11 8 10	12 12 8 12	12 13 10 12		20.0 20.5 24.0 22.5
Average	10.5	11	11.7		
Four vacuoled animals	12 10	14 9	13 13	12 12	20.5 23.0
Average	11	11.5	13	12	

Common two-vacuoled race

FIRST VACUOLE	SECOND VACUOLE	TEMPERATURE
10.7	10.9	20.5
10.2	9.8	22.0
10.0	10.6	25.5
Average....10.3	10.4	

progressive lessening of rate of pulsation from anterior to posterior end. This is not so evident in the records of the two-vacuoled race as both vacuoles appear to beat in almost identical rate. While the observations were not taken at the same temperature, the relative frequency of the various vacuoles in each case would remain approximately the same.

An interesting condition appears in the first table by averaging the average time of contraction of the vacuoles in each group. The result is:

	seconds
Two-vacuoled group	10
Three-vacuoled group	11
Four-vacuoled group	12

There is a steady slowing down of the rate of pulsation as the number of vacuoles increases. Whether this will be borne out by more extensive studies remains to be seen.

1. The effect of salt solutions on the rate of contraction. The work of other investigators has shown that marine protozoa seldom possess contractile vacuoles and that when fresh water forms are subjected to saline solutions the rate of contraction decreases greatly and in some cases the vacuole disappears entirely (6). This race was subjected to salt solutions of various strengths to determine whether it was possible to cause some of the extra vacuoles to disappear permanently. Enough sea salt was added to hay infusion to bring the percentage of salt to 0.06 per cent or to 0.1 per cent. The animals could not withstand an immersion in a solution as strong as 0.5 per cent immediately but they survived very well if the percentage was allowed to increase slowly through evaporation.[1] The salt slowed the contraction rate but did not in any way influence the number of vacuoles. The most marked effect in this direction was while the animals were still unaccustomed to their environment. After they had become somewhat acclimated, to the new conditions after which the rate of pulsation became more normal although it never regained the usual rapidity. This may account in part for the varying results noted below.

	FIRST VACUOLE	SECOND VACUOLE	THIRD VACUOLE	TEMPERATURE	CONCENTRATION OF SALT
				deg. C.	*per cent*
Two vacuoled animals......	25	25		20.0	0.06
	27	23		21.5	0.1
Three vacuoled animals......	34	23	45	20.0	0.06
	14	19	18	21.5	0.5
	26	19	18	21.5	1.0

2. The effect of cold on the rate of contraction. Cold slows the rate of contraction markedly and extreme cold, i.e., 1° to 2°C. apparently brings contraction almost to a complete stop.

3. The volume of the vacuoles of the two races. In making this comparison the volume of the animals was calculated in the following manner. The percentage of difference in weight of a paramoecium modeled in clay and a cylinder of the same length and diameter made of the same material was determined. The volume of a cylinder the length and diameter of a living paramoecium was calculated and the volume of the paramoecium was obtained by reducing the volume of the cylinder by the determined percentage of difference between the clay models.

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[1] It might be noted here that cultures containing a small percentage of salt thrived wonderfully and the infusion seldom became as murky or as bad smelling as the untreated cultures. It seems likely that the salt prevents the great growth of bacteria producing putrefaction, but it seems to interfere in no way with the supply of food for the paramoecia. Animals treated in this way are more tough and when they burst under pressure the protoplasm oozes out in a thick mass instead of the usual thin stream. Frequently the animal is able to clear itself of the extruded mass and the mutilated area is covered over. Under normal conditions the ejection of the protoplasm is so rapid that there is no possibility of this recovery. This treatment might be of assistance with individuals to be operated on.

The diameter of the vacuoles was obtained from a number of fixed preparations some of which were sectioned.

Multi-vacuoled race

Average length	233 μ
Average width	51 μ
Volume	284, 493 cubic μ
Capacity of each vacuole	500 cubic μ (diameter, 10 μ)

To deliver themselves of a quantity of liquid equal to the volume of the animal, at a temperature of 22°C., the

2 vacuoled animals contract 284 times in 47 minutes at a 10 second rate
3 vacuoled animals contract 190 times in 35 minutes at a 11 second rate
4 vacuoled animals contract 142 times in 28 minutes at a 12 second rate
5 vacuoled animals contract 114 times in 25 minutes at a 13 second rate
6 vacuoled animals contract 95 times in 22 minutes at a 14 second rate

The rate for the five and six vacuoled animals has been based on statistics given above (page 309).

Common race. Large individuals

Average length	265 μ
Average width	53 μ
Volume	397, 690 cubic μ
Capacity of each vacuole	664 cubic μ (diameter, 11 μ)

At the same temperature these animals require forty-nine minutes to pump out a quantity of water equal to the volume of the animal.

Small individuals

Average length	180 μ
Average width	53 μ
Volume	194, 260 cubic μ

This lot would require twenty-three minutes for the same operation.

It is evident from these figures that the new paramoecia are superior to the larger forms in the quantity of liquid they can excrete in a given time but in general are not quite equal to the smaller races in this respect. I have been unable as yet to obtain a wild race as large as these new animals to compare their respective resistances and this will form an interesting part of future work. I have not attempted as yet to determine whether there is any correlation between resistance to various substances and the number of vacuoles but the addition of vacuoles in the individuals as the culture ages and the conditions are presumably becoming more unfavorable is a response that is very suggestive.

d. Conjugation

A cycle of conjugation was awaited for over a year and then a method of bringing it about at will was hit upon accidentally. Two cultures which had been started with 200 cc. of hay infusion were allowed to stand uncovered. In seven days the fluid had evaporated to one half and a strong epidemic of conjugation was observed. The experiment was repeated successfully eight times, conjugants always appearing when the fluid had been reduced to approximately one half its original volume. With the containers used this usually required a week.

From these results it seemed probable that conjugation had been produced by the rapid concentration of metabolic products. To test this theory further a number of cultures were set up. Some jars were covered and others were allowed to evaporate. The jars used were much smaller than the original containers and the liquid surface exposed was not more than one third as great. Consequently the evaporation was much slower and three weeks were required to reduce the liquid to the point that one week produced in the larger jars. In three jars that contained (1) pure hay infusion, (2) hay infusion plus 0.05 per cent sea salt, and (3) hay infusion plus 0.1 per cent sea salt, although evaporation did not take place conjugation began in (3) in thirty-six days, in (2) in thirty-eight days and in (1) in thirty-nine days. These cultures had been started in drinking glasses. Three cultures similarly made at the same time were allowed to evaporate. No conjugants appeared. When the medium became very low in the container it was replenished with fresh infusion and again allowed to evaporate. This was repeated five times and no sign of conjugation was observed.

During the year previous to these experiments cultures had been made up using 500 or 600 cc. of liquid and in this time as has been stated no conjugation took place. Conjugation has been obtained in eighteen cultures when the exposed surface of the fluid was sufficient to permit rapid evaporation at room temperature or when the quantity of fluid was small and the multiplication of the animals rapid.

It is of considerable interest to note that when conjugation did take place in cultures to which a minute quantity of sea salt had been added the epidemic did not last nearly as long as in the untreated infusions. In one set of experiments conjugation lasted for twelve days in the unsalted culture while in the culture containing 0.1 per cent sea salt it died out in three days. Another time an untreated medium maintained conjugation for thirty-three days against thirteen and nineteen days in two salt cultures. The length of one of the periods of conjugation is figured on chart 7.

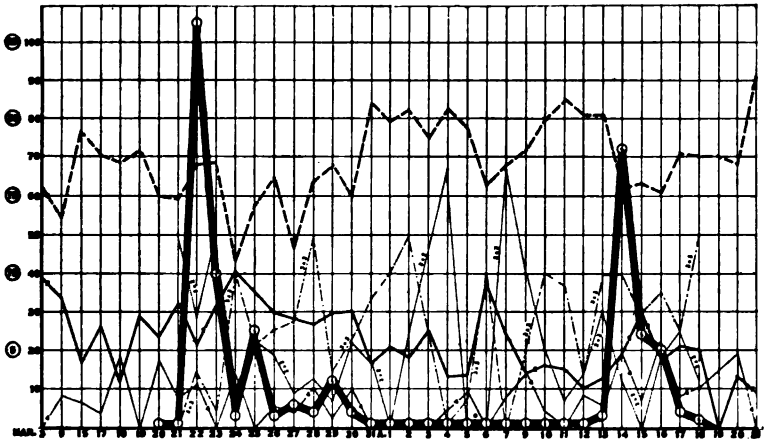

Chart 7 This chart was drawn from records obtained from a single culture and illustrates the percentage of conjugants to unfused forms found during a cycle of conjugation. The figures in the circles at the extreme left of the chart indicate the percentage of conjugants and the heavy black band traces the fluctuations of the percentage of conjugants across the graph. The other curves represent either individuals or conjugants and are labeled on the chart. It will be noted that when the number of conjugants is high the percentage of three and four-vacuoled forms decrease and increase as conjugation dies out.

In brief, conjugation has been obtained in cultures that have been started with single individuals under the following conditions.

1. Conjugation appeared in practically all cases when the culture was evaporated rapidly causing the metabolic products to concentrate considerably.

2. Conjugation appeared in cultures having a comparatively small amount of fluid supporting a large number of paramoecia.
3. No conjugations were seen in some cultures undergoing the same treatment as 1 and 2 particularly when the concentration was not rapid.
4. No conjugation took place in cultures made up with a large amount of fluid.
5. When a slight amount of sea salt was present conjugation sometimes occurred a little before the control of plain hay infusion showed signs of this condition and in all cases the epidemic of conjugation in the saline solutions died out before it did in the pure hay media.

As a discussion of the various theories of conjugation in relation to the results given above is somewhat foreign to the main subject of this paper a full consideration of the phenomena will be published separately.

VI. GENETICS

The account that precedes this section has dealt with the general behavior and inheritance of the contractile vacuoles. In this section will be considered the inheritance of the extra organs for several consecutive generations and the effect of selection and of conjugation on the race.

While these experiments might have been carried on for more generations the technical difficulties were so great as to make further pursuance of this line of investigation unpracticable. In this part of the work the animals had to be examined with the 4 mm. objective in many cases to be certain of the number of vacuoles they possessed, and to do this the animals could be allowed so little fluid to move in that many times I have lost them through drying thereby breaking the continuous line of succession. Indeed, dozens of experiments were started before the results illustrated below were obtained.

a. The inheritance of the contractile vacuoles for four generations

Lines were started with one two, one three and one four-vacuoled paramoecium. As rapidly as they divided the number of vacuoles in the daughter cells was recorded and the animals were separated and again allowed to divide when the operation was repeated. The results of these experiments are shown in the charts 8, 9 and 10. The last generations on chart 8 and 9 show practically 50 per cent of twos and threes. The same generation on chart 10 shows a much higher percentage of twos. A study of these charts will, I think, convince the reader that

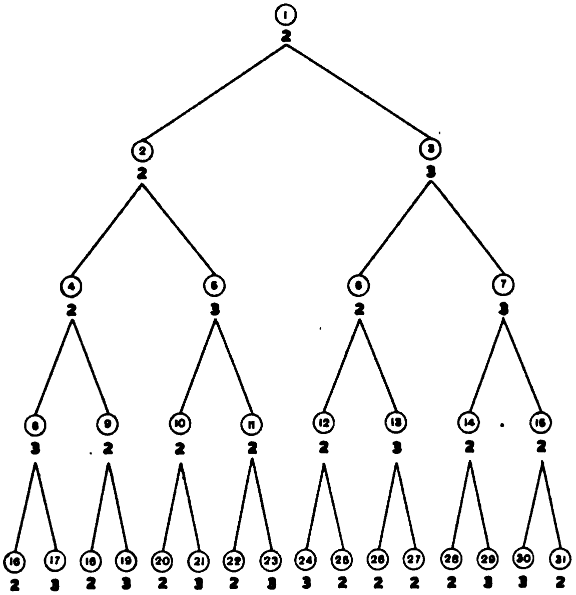

Chart 8 This figure illustrates the mode of inheritance for four generations from a two-vacuoled progenitor. The heavy figures indicate the number of vacuoles for any descendant.

the number of vacuoles any one individual may possess is no indication of its potentialities—that the fewer-vacuoled paramoecia may give rise to animals with more vacuoles and vice versa. The last statement was demonstrated by starting cultures with several of the animals possessing different numbers of vacuoles from the fourth generation of these lines. After the lines had multiplied considerably examination showed the percentages to be in no way different from those given throughout this paper. In the case of experiments such as these the two-vacuoled forms tend to predominate, as fresh hay infusion was used for each generation. As has been pointed out before, fresh medium causes the animals to divide more rapidly and that rapidity of division may be said in a general way to keep the number of vacuoles low.

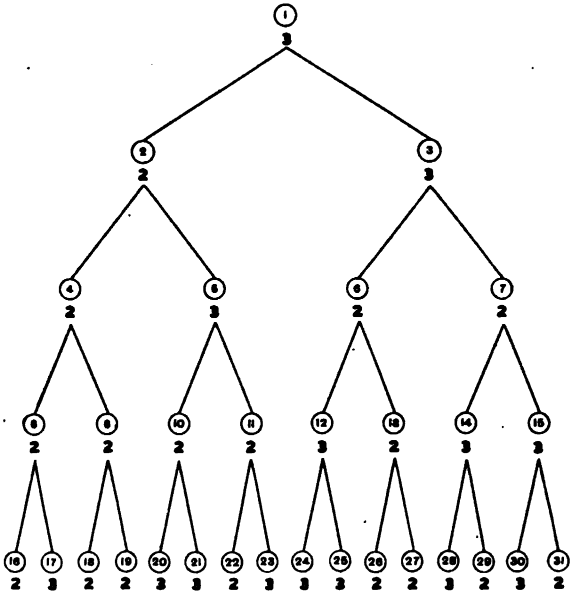

Chart 9 In this case the line was started with a three-vacuoled paramoecium.

b. Effect of selection

Selection has been carried on in three ways.

First method. Individuals possessing two, three, four and five vacuoles were isolated. When they had divided, the number of vacuoles in each was recorded and, in the case of the two-vacuoled line, the paramoecium with the lowest number of vacuoles was allowed to continue while in the three, four and five-vacuoled lines the highest vacuoled animals were always selected. The individuals not selected for continuing the lines were discarded. This plan was carried on for a period of from nine to fourteen generations and the results are given on chart 11. Here the vacuole number of the progenitor is placed at the top of the line in a diamond-shaped frame. Directly beneath it is the number of vacuoles in the selected descendents. To the left of this line is placed the number of vacuoles in the sister cell. At first sight it would seem that selection has some effect but figuring the percentage of animals with twos, threes, fours and fives in each line it is found that they vary little more than 10 per cent.

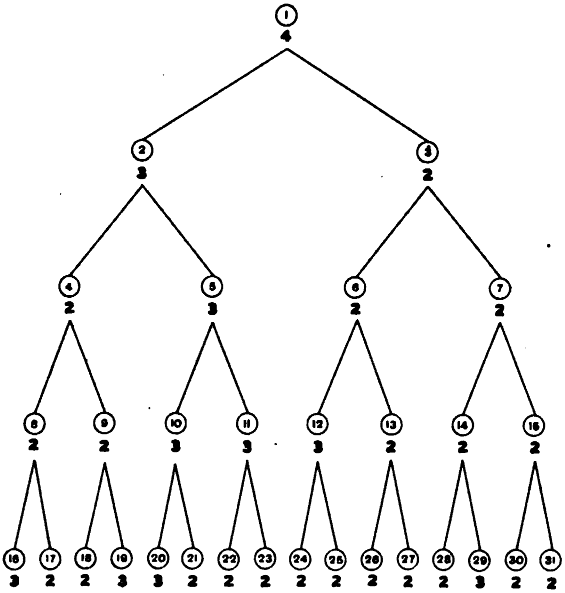

Chart 10 A four-vacuoled paramoecium gave rise to paramoecia possessing the number of vacuoles indicated on this chart.

Second method. The same difficulty was experienced with selecting by the first method as was found in following all the animals for several generations, i.e., the danger of losing an important individual through drying. By the second method each animal was allowed to multiply for a considerable number of generations. A large number would then be examined and the percentage of twos, threes, fours and fives noted. One would then be isolated to start a new line. In an experiment where selection was carried on for nearly three months according to this method at the time of writing no tendency to produce more high-vacuoled animals is evident.

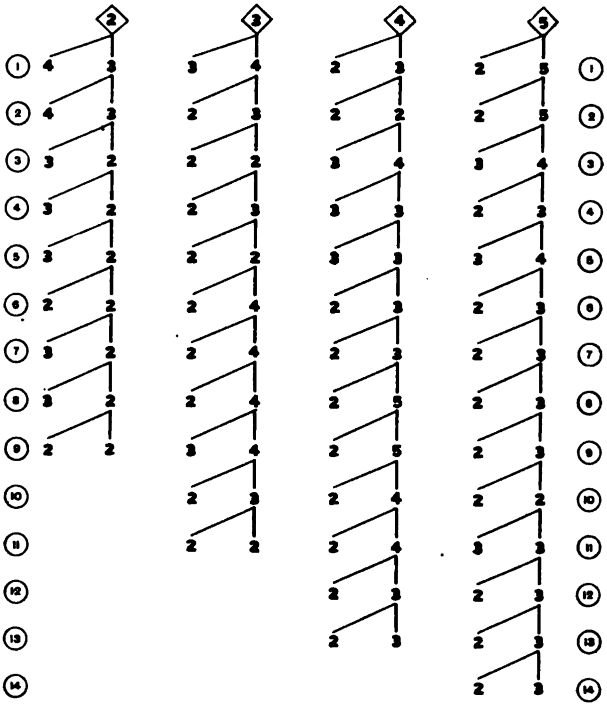

Chart 11 This figure illustrates the effect of selection and is described on page 316.

Analysis of Chart 11

	TWO VACUOLES	THREE VACUOLES	FOUR VACUOLES	FIVE VACUOLES
	per cent	*per cent*	*per cent*	*per cent*
Line 2	53	37	10	
Line 3	50	27	23	
Line 4	40	37	15	7
Line 5	41	41	7	10

Experiment A was started with a two-vacuoled paramoecium and selection was always made of animals with the least number of vacuoles and the line carried on with them. The last examination of this culture showed the percentages to be as follows:

	per cent
Two-vacuoled paramoecia	13
Three-vacuoled paramoecia	78
Four-vacuoled paramoecia	9

Experiments B and C were carried on with selection for animals with the most contractile vacuoles.

Experiment B started with a three-vacuoled paramoecium gave rise to:

	per cent
Two-vacuoled paramoecia	31
Three-vacuoled paramoecia	69

Experiment C started with a four-vacuoled paramoecium produced:

	per cent
Two-vacuoled paramoecia	43
Three-vacuoled paramoecia	57

In Experiment A where the selection was for the animals having the least number of vacuoles the percentage of multi-vacuoled paramoecia produced was greater than in B and C. It might seem that the two-vacuoled animals of Experiment A were possessed of a potentiality to produce more multi-vacuoled offspring than were the paramoecia of B and C. As far as is known the culture conditions in all three experiments were the same. As has been stated several times before, the extensive data that have been gathered on this race under all conditions leads me to feel confident that in experiments such as A the greater number of multi-vacuoled animals were produced because of some characteristic of the culture medium which either slowed down the division rate (allowing more time for vacuoles to develop) or had some other influence on the animals which produced extra vacuoles and which as yet I have been unable to discover. In B and C this factor was presumably absent or was not so potent.

c. *Immediate and temporary effect of location of the vacuoles in the parent form on inheritance by offspring*

When a paramoecium possessing the most common arrangement of vacuoles (the supernumerary vacuoles located posteriorly) divides, the anterior half receives fewer vacuoles than the posterior half. For instance, an animal with three vacuoles—one anterior and two posterior—divides. Just before division a new vacuole is added to each end which results in a daughter paramoecium from the anterior half with two vacuoles while the daughter arising posterior to the constriction has three vacuoles. The paramoecium springing from the posterior half starts its life cycle with more vacuoles than does its anterior mate but the anterior animal, however, as had been emphasized before, has not lost the power of producing paramoecia with the higher number of vacuoles.

d. *Effect of conjugation*

When conjugation was first observed it seemed that there was what might be termed a selective mating or more exactly that there were more two-vacuoled animals conjugating at the beginning of the epidemic than multi-vacuoled individuals. Further work and the final plotting of the results showed that this was not the case. Neither was there a tendency for threes to pair only with threes or fours with fours but there was a heterogeneous mating in approximately the same percentage as the frequency of the various number of vacuoles in the individual units. Chart 12 illustrates these points. Conjugants were found paired in nearly all possible combinations: 2 × 2, 2 × 3, 2 × 4, 2 × 5, 3 × 3, 3 × 4, 3 × 5, 4 × 4 and 4 × 5. During an epidemic of conjugation the proportions of these various combinations on any one day followed in a fairly close manner the percentage of single animals having two, three and four vacuoles in the same culture. This relation becomes clearer when graphically represented as on chart 12. Here the lines representing the various combinations in conjugation may be seen to follow rather closely the rise or fall of the lines representing the vacuole number in the single paramoecia. More of the multi-vacuoled paramoecia conjugate at the end of the epidemic than at the beginning, apparently, not because of different physiological characteristics but because more of these forms appear as the culture ages.

The period of conjugation was awaited with great interest as furnishing an opportunity for determining the nature of this

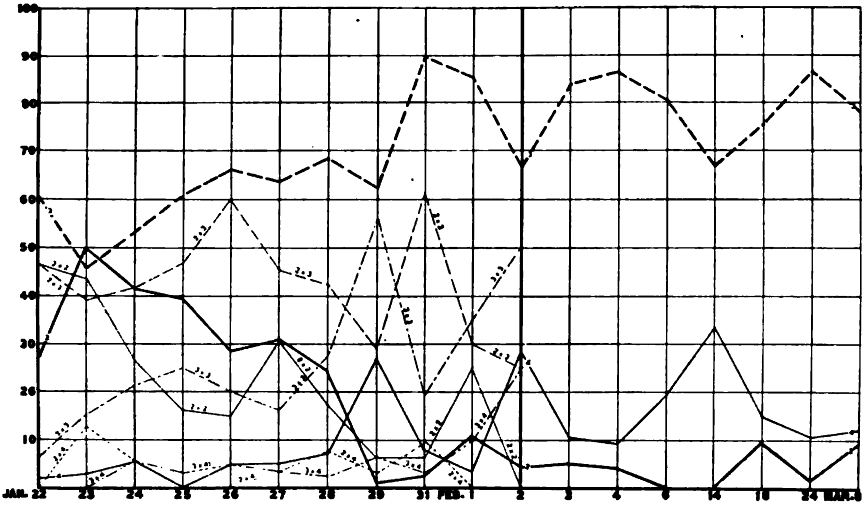

Chart 12 This graph is reproduced to show the manner in which the various combinations of paramoecia in conjugation follow closely the percentages of two, three and four-vacuoled paramoecia on any day. Note that as conjugation ceases on February 2 the percentage of three and four-vacuoled paramoecia increase. Further discussion is to be found on page 320 under "Effect of Conjugation."

new character. If the paramoecia were homozygous then we should expect no change in the inheritance of the potentiality for extra organs. But should they be heterozygous, individuals might result which were pure for the lower number of vacuoles. I have used the words 'might result' in the last sentence, since, as far as we know, conjugation between heterozygous paramoecia would present equal possibilities for the offspring of the exconjugants to be heterozygous as it would for a homozygous line to appear. However, this line is evidently pure for this character since exconjugants when separated produced offspring which showed no variation in the percentage of vacuoles from the number possessed before conjugation began as is indicated by the following observations. Single conjugants of the various combination of vacuole number were isolated and allowed to separate and divide a number of times and then examined. The results were not different from those obtained on unconjugated forms. To take two typical cases in which the conjugants were isolated, and allowed to separate and divide for a week or more.

	per cent
Conjugant 2 × 3:	
Two-vacuoled paramoecia	31
Three-vacuoled paramoecia	69
Conjugant 2 × 4:	
Two-vacuoled paramoecia	32
Three-vacuoled paramoecia	69

The number of two-vacuoled forms here is slightly higher than the average due probably to the rapid division stimulated by fresh hay infusion.

In one instance the exconjugants were allowed to divide twice, producing eight animals in all, and then each animal was isolated in a separate watch crystal and allowed to multiply for some time. This was done to determine whether any difference in potentiality would crop out due to the redistribution of the micronucleus. No change was observed.

No effect can be ascribed to conjugation other than a temporary increase in the percentage of two-vacuoled forms and a corresponding decrease of the threes and fours. This may indicate that conjugation speeds up the division rate somewhat as it has been shown before that increased rate of division results in individuals with fewer contractile vacuoles than is the case when the division rate is slow.

Chart 5 beginning with March 19 when conjugation began in cultures 1, 3 and 5 shows a slight rise in the 'two' line (marked 1–3–5) and a fall in the 'three' line for the same cultures both, however, returning to very nearly the same point on April 6 as they were on March 15. Conjugation had by this time dropped to less than 0.5 per cent of the population.

e. Attempts to cross the two races

One of the most interesting points to be developed in connection with this new race of paramoecium is the effect of crossing with the common form. This is the first time to my knowledge that two forms of protozoa with definite individual characters have been available for crossing. I have made one hundred and fifty attempts to cross the two forms with no success. The difficulties that beset the mating of these two paramoecia are many. Cultures of both races have to be entering into a period of conjugation at the same time. Single individuals from both races must be placed in as small amount of fluid as possible so that they will have every opportunity of coming together. The chances that either or both of the mated paramoecia are in a condition which makes conjugation necessary are small. The matings must be made up fresh at least, every twenty-four hours for when either of the individuals divide there is no way of telling to which race an animal belongs. In spite of these obstacles I am confident that the crossing will ultimately be achieved after sufficient number of matings have been made and perhaps after certain methods of technique have been determined which are not evident at present.

VII. CYTOLOGY

My studies on the cytology of these paramoecia are not sufficient as yet to permit a detailed account. There is one micronucleus present (fig. 15) which indicates that this race is a variety of Paramoecium caudatum. In all the preparations made I have had great difficulty in staining the micronucleus and in the majority of cases so far I have been unable to make the stain remain in it. The depression in the macronucleus where the micronucleus usually lies is frequently visible but it appears empty. I do not believe that this appearance is due entirely to faulty technique, as the same methods give excellent results when used on the common race.

VIII. DISCUSSION

In the course of some experiments by Dr. M. H. Jacobs on the effect of high temperatures on paramoecia, various lots of these animals were ejected into hay infusions at temperatures ranging from about 36° ot 42°C. Those that survived this treatment were thrown into a battery jar containing hay infusion. In this jar the new animals were discovered. I am still of the opinion (stated in my preliminary note in Science (2)) that these new paramoecia may have been produced as the result of the high temperature, although I have been unsuccessful as yet in a number of attempts to reproduce this race artificially. It would be a most striking coincidence if this multi-vacuoled character has been in existence for any length of time that its discovery should be delayed until found in the culture made up of the descendents of heat treated animals.[2]

In this new paramoecium we have a form which lends itself to a study of the processes of heredity in the protozoa. The character is seemingly not at all comparable to those reported for Difflugia by Jennings (5). Since the vacuoles are, however, very definite structures and a marked variation from the common form, their study and comparison is exceedingly interesting and offers many possibilities. The data possessed at present are insufficient to make a discussion of these hereditary processes of value. Selection experiments, though incomplete, seem to indicate as far as they have been carried that the character cannot be affected by selection in either direction. It will be impossible to determine whether the tendency or potentiality for extra vacuoles is germinally controlled until we have succeeded in crossing the two strains. There is some evidence at present that points to the cytoplasm as the portion of the cell that controls the production of the vacuoles since their production seems to be controlled by so many conditions. Whether these conditions act on the cytoplasm directly or on the cytoplasm through the nucleus there is no possibility of determining with the knowledge available at present.

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[2] Since this paper was sent to press I have received Shumway's paper (11) in which he has reported the formation of extra contractile vacuoles in thyroid fed animals. None of his specimens had more than three vacuoles and the inheritance of the extra vacuole in the form studied has not been demonstrated. The percentage of extra-vacuoled animals among his thyroid fed paramoecia (20 to 30 per cent) is considerably less than I have found in my race (77 per cent).

IX. SUMMARY

1. This race which is a variety of Paramoecium caudatum is considered possibly to have arisen under experimental conditions when certain animals were subjected to high temperatures.

2. The animals of this race range in length from 167 μ to 307 μ and are unusually strong and resistant.

3. A slightly denser portion extends across the middle of some of the individuals as a band.

4. The extra vacuoles are generally located in the posterior end of the cell.

5. The number of vacuoles possessed by individuals of this race varies from two to seven.

6. The number of vacuoles may increase during the life time of the individual.

7. Various conditions affect the number of vacuoles present in the individual, such as the rate of division and the age of the culture medium.

8. Though extra vacuoles may not appear for several generations for the above reasons, the potentiality for this organ has not been lost.

9. The division rate ranges between one and five times in twenty-four hours.

10. Besides being influenced by the rate of division the vacuole number may apparently be increased in the individual by the presence of katabolic products in the environment.

11. The resistance of this race to heat, cold, distilled water, etc., is very high.

12. The rate of pulsation of the vacuoles is slightly slower than is that of the vacuoles in the common race but the new animals are in general superior to the common race in their powers of excretion.

13. Conjugation is believed to be caused by the concentration of katabolic products in the environment.

14. It may be said that a potentiality for *extra* contractile vacuoles rather than for a definite number of the organs is passed on from generation to generation and that this potentiality if effected by various conditions during the life time of the individual.

15. Selection has no effect in raising or lowering the vacuole number.

16. The location of the vacuoles in the parent has an immediate but temporary effect on the number possessed by the offspring.

17. Conjugation has no effect upon this new character.

X. BIBLIOGRAPHY

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PLATE 1

EXPLANATION OF FIGURES

The figures are reproduced at a magnification of 480 × with the exception of figure 16 which is 1120 ×. Figures 3, 4, 5, 6 and 8 were drawn from living animals slightly flattened under pressure. The others were outlined from fixed preparations.

In preparing these figures an attempt has been made to reproduce all the various characteristics of the contractile vacuole observed. In general when the vacuoles are expanded the radiating canals are not visible but in favorable specimens or in animals under pressure they appear as thread-like rays as shown in figures 2, 3, 4, 5 and 10. Note that in most cases the vacuoles lie in a line parallel to the outline of the body. The anterior end of the paramoecium is toward the upper part of the page in all the plates. In all the figures the contractile vacuoles are not stippled.

1 Paramoecium with three contractile vacuoles arranged in the most common manner.

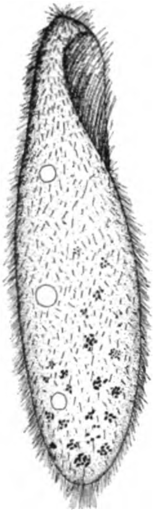
2 Paramoecium with two vacuoles.

3 Paramoecium with extra vacuole in the anterior end—a rare grouping.

4 Paramoecium with four vacuoles in the usual position but with one vacuole slightly displaced to the right.

5 Paramoecium with four vacuoles in common arrangement.

6 Paramoecium with four vacuoles in the unusual grouping of two in the anterior end and two in the posterior end. Note the slightly darker area across the middle of the animal.



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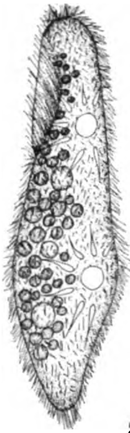

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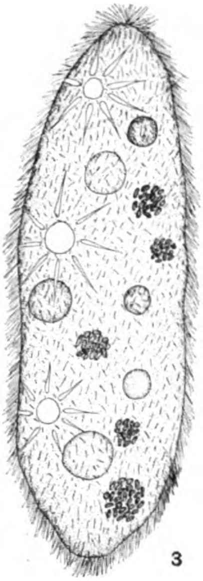

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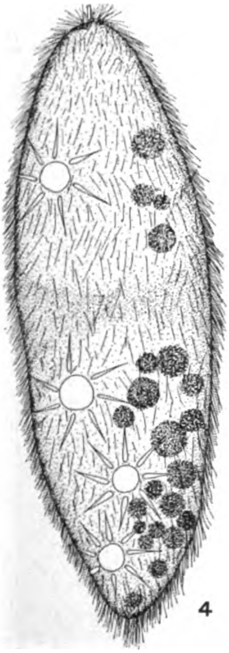

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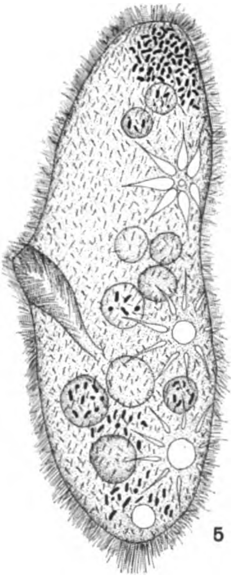

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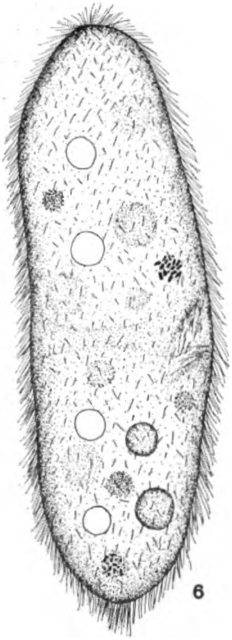

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PLATE 2

EXPLANATION OF FIGURES

7 Paramoecium with five vacuoles.

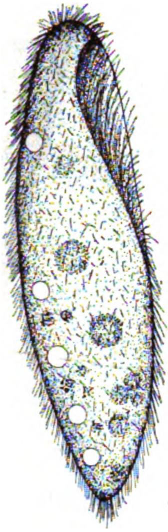
8 Paramoecium with five vacuoles two being located in the anterior end.

9 Paramoecium with five vacuoles, two of which are slightly displaced to the right. This displacement is rare.

10 Paramoecium with six vacuoles.

11 Paramoecium dividing. Before constriction occurred this was a three-vacuoled animal. A new vacuole has been added to each half.

12 Paramoecium with seven vacuoles.



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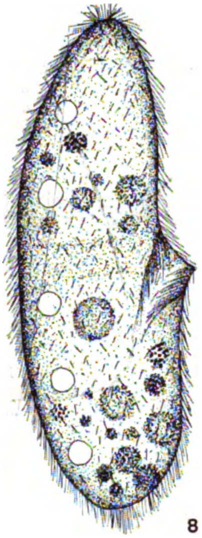

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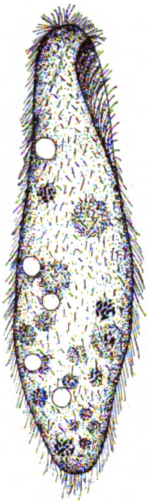

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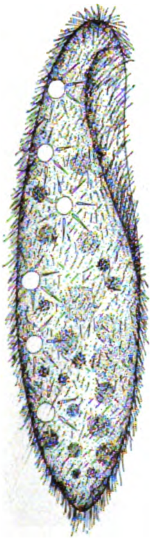

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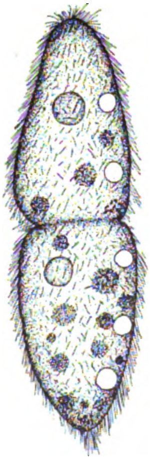

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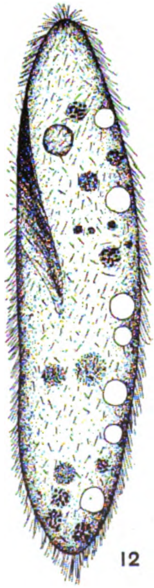

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PLATE 3

EXPLANATION OF FIGURES

13 A paramoecium dividing. This animal before division had four vacuoles arranged as in figure 6. A new vacuole has been added to each end.

14 A conjugating pair of paramoecium. One animal has three and the other two vacuoles.

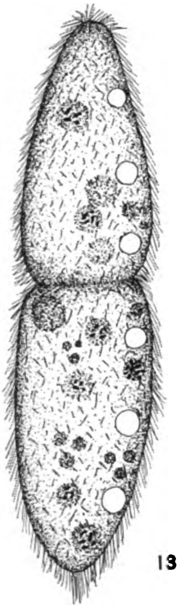
15 Drawn from a fixed and stained preparation showing the macronucleus and the single micronucleus.

16 Drawing made with the aid of an oil immersion lens showing the vacuoles to be surrounded by a morphological membrane.

17 A paramoecium dividing. Before division began this animal had five vacuoles arranged as in figure 7. A new vacuole has been added to either end.

18 A paramoecium with a greatly enlarged vacuole. This condition has been rare in my cultures. Occasionally the animals recover from this condition but more frequently they die with the vacuole still distended.

19 The mouth of this paramoecium has been forced out under pressure.



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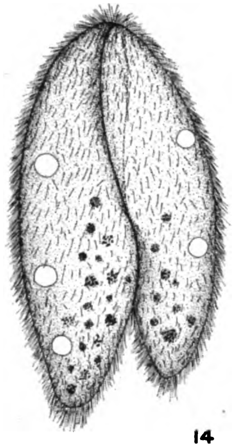

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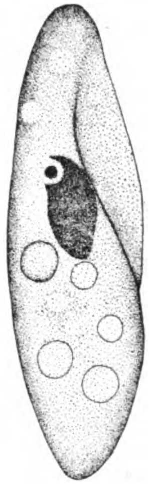

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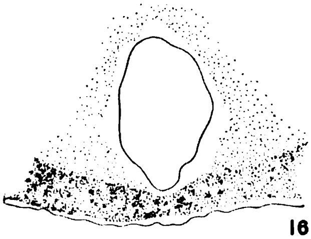

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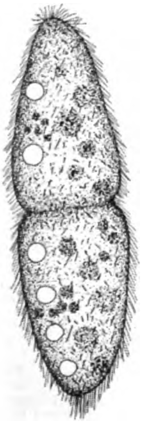

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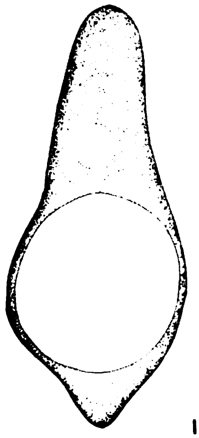

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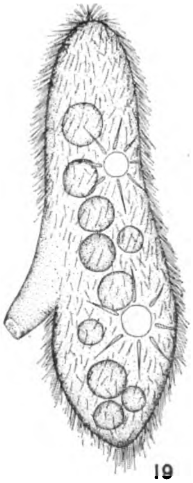

19

[Reprinted from THE AMERICAN NATURALIST, Vol. LI., September, 1917.]

AN ATTEMPT TO MODIFY THE GERM PLASM OF ŒNOTHERA THROUGH THE GERMINATING SEED

WHEN a new character appears in a homozygous race or species it may be either a mutation or an acquired character. If a mutation it has been produced because the germ plasm has in some way been affected and the succeeding generations may be expected to show the same variation. If an acquired character it will be present for a single generation and is then lost unless the cause that produced the somatic change also modified the germ plasm in such a manner that it may develop the same character, in succeeding generations. By "acquired characters" is meant any and all changes that are wrought in the soma of the organism by the environment considered in its broadest sense. It is the creed of modern biology that acquired characters are not inherited unless the environmental influences also play on the germ cells even while focused on the body tissues, producing at the same time potential alterations in the former and visible changes in the latter.

The causes of mutation are in dispute. It is fairly obvious, however, that in the reported instances of mutation the variations arose as the result of changes in the germ plasm. A mutation, therefore, has its origin from within and this origin has no very evident connection in any way with external conditions. If a case of an acquired character is shown to be inherited it is clear that the germ plasm of the organism must have been affected. The stimulus to change, therefore, in contrast with the cause of mutation, would have come from without. While the end result—the alteration of the germinal constitution—is the same in both cases, the method or cause by which it is brought about is different. From this point of view, the relative value in evolution of mutation and of hereditary acquired characters is open to various interpretations. I think that in the case of mutations it may soon be possible to demonstrate that some of the so-called examples of "mutations" are due to or are associated with irregularities of karyokinesis. It is not at all inconceivable that outside conditions producing acquired characters may at the same time effect chromosome structure and behavior in the germ plasm. The cause for unusual activity on the part of the chromosomes in either case may, however, well be totally different.

Interesting as are the effects of natural influences on the germ plasm, of greatest importance is the problem whether or not modifications may be produced and controlled artificially. The fact that experimental research is tending to show the specificity of certain chemicals for various organisms or parts of organisms makes hopeful the outlook for finding a specific or many specifics that will act upon the mechanism of heredity or different parts of that mechanism. At present our investigations can only be empirical, trying this or that method more or less blindly. Considerable work in this field has already been done. Without entering into a full discussion of the numerous investigations, it may be said that alcohol, temperature, humidity, ether, zinc sulphate and radium have been used in attempts to alter the germinal constitution. Changes indeed have been produced in some cases but the effects have been generally physiological in nature either interfering with development or influencing color, length of hair, etc. In all cases where the offspring resembles the altered parents the results have been readily interpreted on the assumption that eggs or embryos are influenced at the same time as were the forms producing them. MacDougal reports having produced changes in one of the œnotheras through ovarial injections. Although it may be possible that he produced modifications, the plant he selected for experimentation was unfortunate since the natural variability of the œnotheras is in most cases great, and the gametic purity of his material was not clearly demonstrated.

In many ways plants offer the most favorable material for the study of experimental variation. Many parts of the plant are adapted to experimentation. The pollen may be subjected to treatment, the ovaries may be injected with chemicals or otherwise handled, the seeds offer themselves to various manipulations, many stages of growing plants are available for experimentation, a variety of experiments may be conducted and, if there are numerous branches, controls may be maintained on the same plant. Coupled with these advantages are possibilities of cultivating pure lines for many generations. It would be difficult indeed to find an animal about which all of the above statements could be made.

Two methods of inducing germinal variation seem practical. The first is the treatment of the germ cells. MacDougal's ovarial injections lie in this class. The other method is to attack the buds or growing points of the stems. I do not at present believe that exact results can be expected from ovarial injections since the ovules are generally so tightly packed that fluids can not readily circulate and it is impossible to know the quantity and the strength of the fluid that may reach the germ plasm. The treatment of pollen may offer greater possibilities unless unforeseen technical difficulties are encountered. By subjecting growing points to treatment the germ plasm may more readily be affected and the complete chromatin equipment may be placed under the influence of the materials used. If germinating seeds or seedlings are immersed in a given chemical solution we may have reason to expect that the cells of the growing points are probably in contact with the solution or some derivative of it. If the chromosomes of a growing point can be influenced it is possible that the organs that are developed from this point may be altered.

Two years ago, in connection with a program of study dealing in general with problems of development, I had the opportunity of making some experiments on seeds and seedlings of *Œnothera biennis* L. (the Dutch *biennis*) from a pedigreed line which had been inbred for at least eight generations. *Œnothera biennis* L. is one of the most stable species so far studied in this genus and its rare "mutations" are known from the research of Professor DeVries. Consequently it seemed justifiable to anticipate that any results obtained through experimental treatment may readily be recognized. To Dr. Bradley M. Davis I am indebted for suggesting *Œnothera biennis* as a favorable plant for study, for many pedigreed seeds and for the complete freedom of his garden. Without his aid in the study of the plants and without his advice I should have made little progress. In preliminary studies of this sort all the in-investigator can hope to accomplish is to determine certain solutions which produce suggestive effects. The results of my studies are here brought together in hope that they may be of some help to other workers.

As has been pointed out, studies of this character must at present be largely empirical. The problem is to find chemicals that will modify the structure of the germ plasm or bring about irregularities in the distribution of the chromosomes. Some of the chemicals used are those frequently employed in fixing fluids; others were selected for various reasons.

As shown in the List of Experiments the seeds and seedlings were soaked for varying lengths of time in the solutions. The material was either thoroughly washed before being placed on moist filter paper in petri dishes to complete germination or it was placed on paper which had been moistened with the same solution as that in which the material had been soaked. Since the seeds of *Œnothera biennis* are about 96 per cent. viable, seed sterility was not an important factor in the results of the experiments. Dr. Davis's large cultures under normal conditions were used as controls. About one hundred seeds were used in each experiment.

LIST OF EXPERIMENTS

Fluids	Percentage	Seeds or Seedlings	Time in Solution	Percentage of Germination
Acetic acid	0.125	Seeds	4 days	25
	0.125	Seedlings	3 days	
	0.625	Seeds	29 days	40
Butyric acid	0.25	Seeds	34 days	40
	0.5	Seeds	34 days	0
	1.0	Seedlings	1 day	
	1.0	Seeds	5 days	0
Chloral hydrate	0.75	Seedlings	5 days	
	0.75	Seeds	34 days	50
	0.375	Seeds	24 days	0
	0.187	Seeds	24 days	50
Chromic acid	0.03	Seedlings	11 hours	
	0.015	Seeds	4 days	65
	0.015	Seeds	3 days	60
	0.015	Seedlings	21 days	
	0.015	Seeds	3 days	60
Ethyl alcohol	5.0	Seedlings	8 days	
	5.0	Seeds	27 days	0
	1.0	Seeds	18 days	40
	0.5	Seeds	3 days	60
	0.5	Seeds	24 days	80
	0.25	Seeds	24 days	A few germinations
Methyl alcohol	1.0	Seeds	18 days	Normal germination
Amylic alcohol	1.0	Seeds	18 days	0
Butylic alcohol	1.0	Seeds	18 days	0
Propylic alcohol	1.0	Seeds	18 days	0
Zinc sulphate	10.0	Seeds	18 days	0
	5.0	Seeds	18 days	A few germinations
Strychnine		Seeds	10 days	0
Pot. bromide and iodide		Seeds	10 days	0
Ferric alum	4.0	Seeds	10 days	0
	0.5	Seeds	14 days	19

RESULTS

In the acetic-acid solutions mold grew vigorously and possibly interfered with the growing plants. The percentage of germination was low. In the young plants the cotyledons were rather more pointed than normal, although this modification was not marked. The leaves of young rosettes also appeared more narrow and pointed, but these peculiarities disappeared as the plants matured.

All the seeds and seedlings treated with butyric acid died.

Chloral hydrate produced no effect other than retarding the period of germination, reducing its percentage, and weakening the plants.

Chromic acid produced by far the most interesting results. In the various solutions used germination was prompt (about the usual three to four day period) but the percentage was materially lowered. The seedlings produced were vigorous in appearance, although the root system was in most cases stunted. There was a slight though not, I believe, significant modification of the cotyledons which were somewhat less pointed than in the type. Some of the seedlings were bright red and practically all had a reddish or pinkish tinge. Growth after planting was slow but all the plants finally developed normally.

Ethyl alcohol produced no modification of structure, although I believe that it will be worth while to continue this line of experimentation. In all cases where seeds were allowed to soak in ethyl alcohol the solution became thick and gelatinous from a substance extracted from the seeds. The percentage of germination was much reduced.

Methyl alcohol retarded germination but the resulting plants were fairly normal.

Amylic, butylic and propylic alcohols all inhibited germination in the strengths employed.

Germination was also inhibited by the solutions of zinc sulphate, strychnine, potassium bromide and iodide and by four per cent. ferric alum.

In general it may be said that the treatment of seeds and seedlings in the experiments has resulted, as in the experiments of others, in reducing the percentage of germination or in a general weakening of the plants rather than in specifically modifying the germinal constitution. The results from the experiments with chromic acid and possibly with chloral hydrate and ethyl alcohol suggest the desirability of further studies. In future work the concentration of the agent and the length of treatment should be studied in greater detail.

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UNIVERSITY OF PENNSYLVANIA,
June, 1917

UNIVERSITY OF PENNSYLVANIA

THE DIPLOID CHROMOSOME COMPLEXES OF THE PIG (SUS SCROFA) AND THEIR VARIATIONS

ROBERT T. HANCE

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE
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ROBERT T. HANCE

Zoological Laboratory, University of Pennsylvania

TEN PLATES AND FIVE TEXT FIGURES

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INTRODUCTION

The terms spermatogonial and somatic have been frequently used synonymously, with respect to the diploid chromosome number, on evidence considerably more theoretical than direct. As a matter of fact comparatively little work has been done on the body cells, as the germ cells, because of their genetic significance, have, until recently, largely held the interest of cytologists. Important as is the theoretical significance of the chromosomes of the germ cells, it seems to me that the chromosomes of the body are fully as interesting. In the soma, as the various cells differentiate in to the tissues that constitute the mature plant or animal, we can see in operation the chromatin whose maturation phenomena we trace through the reproductive cells. What, we may ask, is the behavior of the chromosomes in development? Are they immutable, or do they undergo change? If changes occur, are they correlated with the character of the tissue in which the variation is found? Also in the event of change what bearing does it have on the theory of the individuality of chromosomes?

Four years ago I selected the pig for studying the problems suggested above. The methods of fixation, then in common use, proved hopeless, as the chromosomes, following the use of the usual fixatives, were clumped together beyond hope of recognition—a condition quite typical of the majority of published figures of mammalian mitoses. My work was consequently delayed until a method was discovered which preserves the chromosomes in a condition comparable to the best cytological preparations. The results obtained with this technique (Hance, '17 b) are considered to be representative of the living structures as they compare favorably in every way with those obtained on the grasshoppers, the fixed tissue of which has been compared and checked with studies on living cells. The methods of fixation which have been developed may now be said to place mammalian tissues in the list of workable cytological materials. Probably not the least of the reasons for the few ventures in to the field of somatic cytology has been a scarcity of divisions, but this is an avoidable condition for tissues may be found showing as many mitotic figures as the best testicular material. The difficulty that investigators frequently met with in locating dividing cells in mammalian testes is entirely obviated by securing material from several animals and then selecting for study that animal which is in what I have termed elsewhere, a 'cycle of division.' The cycle holds for both testes and embryo, and I have found it also in plants.

Cytologists, who have studied the cells of the soma, may be be divided into two general groups—those who maintain that the somatic chromosome number is fixed and identical with that of the spermatogonia, and those who believe that it is variable. I believe that it may safely be said that both groups have drawn conclusions from too little evidence and, in some cases at least, this evidence has been obtained from very poor material. This is particularly true of the 'variable chromosome group,' among whom belong those, who, with Della Valle, hold the chromosomes to be unimportant structures and who believe that the supposed variations express and emphasize the lack of chromosome importance. Which of these groups is right will be considered later. Ina recent paper (Hance '18) it has been pointed out that

> only when the investigator of somatic chromosomes has a sufficiently large and properly preserved number of somatic figures to study and has subjected the chromosomes to every possible analysis is he justified in taking up the cudgels for or against the theories that have developed around the many excellent studies on germinal complexes. There is a very apparent and regrettable tendency of late on the part of some cytologists to attempt merely to correlate their own findings with those of some classic work on another form, passing, at times, variations that have been found, either because they were thought unimportant, because they were supposedly pathological, or perhaps,

because they appeared impossible of interpretation. Since the physiological character of the chromosomes are as yet undetermined and since at present our only method of forming a conception of the activities of the chromatic material is through morphological studies in the broadest sense, it scarcely needs to be pointed out that uncritical wotk of the sort discribed is not likely to be productive of results.

It is quite true that when we have determined the behavior of somatic chromosomes we are not a great deal nearer the solution of development for it seems quite evident that the ultimate analysis of this problem must be largely a chemical one. But, since chemical studies of such nature are impossible at present, morphological investigations may indicate the lines which future research may advantageously follow and even may indirectly, through a careful study of the division phenomena in many tissues of a large number of plants and animals, lead to an understanding of the physiology of the cell.

TECHNIQUE

Preparation of tissue. Probably more inaccuracies in cytological work have resulted from the use of poorly prepared material than in any other way. This is particularly true of mammalian studies, and, with the possible exception of the investigations of Winiwarter, the work of other mammalian cytologists is practically valueless and will have to be carefully repeated. We have definite criteria now with which to judge our preserved material, namely the studies on the living cell (Lewis and Robertson, '16). Consequently the cytologist who attempts to unravel clumped chromosomes and to draw conclusions on the supposition that he knows the chromosomes as they are, not only invites destructive criticism of his work, but adds nothing to our knowledge and wastes our time.

The method that has given excellent results has already been described at length (Hance, '17 b). In brief it is as follows:

1. Obtain fresh specimens from as many different animals as possible so as to be sure of obtaining one or more in a 'cycle of division.'

2. Place small or finely teased pieces of fresh tissue into cold Flemming's (strong) solution to which a little urea (one-half per cent) has been added. When the bottles of fluid are surrounded with ice the temperature of the fixative is about four degrees Centigrade. Allow the tissue to remain in the fluid twenty to twenty-four hours. (Flemming's weak solution was also used successfully. See below.)
3. Wash in water for about twenty-four hours.
4. Dehydrate by very gradual steps.
5. Clear from 95 per cent alcohol in cedar oil followed by xylol.
6. Imbed in paraffin.
7. Cut the sections ten micra thick.
8. Bleach the sections for from one to twenty-four hours in peroxide.
9. Stain with iron alum haematoxylin.

This method has never failed to give good results and has been used on over sixty lots of material taken from seven mammalian species. The same results were obtained on testes, ovary and embryo.

I have studied testes obtained at the time of castration from pigs about four or five months old representing three breeds, Berkshire, Jersey red and Poland china. The first two were obtained at Philadelphia and the last from Kentucky. None of the pigs were thoroughbreds, although I was told that the Poland china was 'nearly so,' whatever that may mean. Forty-two embryos of various sizes have been fixed either entire or teased. The fixation in the case of those killed without teasing is as good as in the shredded embryos. Only a few of the specimens from this lot of material have been studied, since it was thought better to initiate this study with thorough observations on a small number of animals. It has been a matter of considerable regret that I have been unable to study any ovarian chromosomes. I recently prepared embryonic ovaries taken from foetuses about half grown, but all the material proved worthless, as the chromosomes were badly clumped. The only explanation I have to offer for this is that the system in the Philadelphia slaughterhouse in which the pigs were obtained was rather slow and the embryos were probably dead before they reached me.

Since the work on sectioned material was completed I have obtained another lot from Cincinnati. These specimens I preserved both in Flemming's weak and strong solutions to which urea had been added. The fluids were used at a temperature of four degrees Centigrade. The embryos apparently were not in a cycle of division for figures were too few to justify study. The amnion from the same pigs was fixed separately and mounted without sectioning. The amnion is quite thin and gave excellent results with the certainty of uncut cells. The slight shrinkage which occurs during the infiltration of paraffin is avoided and the chromosomes appear slightly larger in the amnionic material and in general, better separated. No differences were noted in the character of the fixation by either killing fluid. Both gave excellent results as may be judged from the photomicrographs, figures 88, 89, 90 and 93. I cannot say, at present, whether the weak solution will work equally well on thicker tissue. The chief advantage of the weak solution, as far as known now, is one of cost.

Counting and checking

When as large a number of chromosomes are involved in as small a space as are the chromosomes in the cells of the pig, drawing and counting is not easy, notwithstanding the almost perfect separation of the individual elements. Inaccuracies in drawing were avoided in the following manner: The chromosomes of a cell were carefully drawn on a 3 x 5 card and the drawing was then checked with oculars of various powers. The card was then filed away for a time. Later, it was brought out and on it the same set of chromosomes was again drawn. This drawing was checked as in the case of the first. Then the two drawings were compared and the points of difference, should any be found, were decided by comparison with the cell under the microscope. This method saved considerable time and, I believe, gave very accurate results.

Methods of measuring

The chromosomes were enlarged with a pantograph six diameters over the original magnification (which was 3400 ×). This not only made easier to measure the chromosomes but gave somewhat more accurate results. Many of the chromosomes are practically straight, so that a rule could be used in measuring. The automatic map measure was employed in obtaining the length of the curved chromosomes. The advantages of this instrument have been described (Hance, '18). It has the disadvantage that millimeters must be estimated. This, I believe, I have been able to do with an error of at the most not more than one or two millimeters. With the large magnification of 20,400 × these results appear to me to be sufficiently accurate.

Mechanical aids

The automatic tally register has been found a great convenience for counting the chromosomes. It enables the investigator to go from the drawing to the microscope without the necessity of remembering the number that has already been counted. The chromosomes are, of course, checked as counted. Before this device was tried drawings would frequently have to be counted several times to be certain that no error had been made.

An adding machine has been of the greatest assistance in this work as has also a 'Calculex,' or circular slide rule, which was used for determining percentages.

A large number of drawings of mitotic figures are presented with this paper, partly to counteract the general impression, gained from published mammalian studies, that clear figures are difficult to find, but largely to give a better idea of the appearance and relationship of the chromosomes of the various cells.

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The many facilities placed at my disposal by Dr. McClung, and his constant interest and friendly advice and criticism have made my stay at the University of Pennsylvania not only profitable, but very enjoyable, a period always to be remembered with great pleasure.

I feel that a list of acknowledgments would be incomplete if no mention were made of my appreciation of and indebtedness to my mother and father to whose encouragement, constant interest and sympathy I owe so much.

THE CHROMOSOMES OF THE GERM CELLS

The diploid chromosome number

Only those cells have been considered in which the chromosomes were well separated (figs. 1 to 20) where the count could be made with comparative ease and accuracy. Forty chromosomes have been found constantly in the spermatogonia of the pig. This number has appeared in testicular material from three Berkshires, five Jersey reds and one Poland china, the material being gathered from rather widely separated regions of this country. The drawings in figures 1 to 20, though selected for flatness to show the full length of the chromosomes are not exceptional as to clearness. The photomicrographs in figures 88 to 99 emphasize the separation and sharp outlines of the chromosomes.

It may occur to the reader that the chromosome number may be influenced by precocious division, since, in the drawings, splits are shown in the chromosomes while they lie in the metaphase plate. With experience the chromosomes which have divided are distinguishable by the difference of level and diameter. Furthermore, the count of forty has been made in cells in prophase (figs. 1, 2 and 3) and also in anaphase (fig. 16). With the counts in these three stages checking, I feel confident of the accuracy of the result.

The structure of the chromosomes

Figures 1 to 20 show that there is some variation in the width or diameter of individual chromosomes, in any given cell. The difference within the cell is very slight and may be due to the fact that the dye is extracted more rapidly from small than from larger chromosomes. In more heavily stained material this difference is not so obvious (figs. 4 and 5) but exists to some extent. The prophases do not show this variation in diameter. In some of the metaphase chromosomes I am inclimed to believe that the extra width is due to the beginning separation of the chromatids, the split being filled with dye. It will be noticed, however, that the diameter of any one chromosome is the same throughout its length.

The presence at various stages of splits in the chromosomes is so variable that at present definite conclusions cannot be drawn. In material passed through paraffin splits are occasionally visible in a few chromosomes in prophases (fig. 3) and it is not possible to determine whether these are obliterated before metaphase is reached or not. From studies of unsectioned amnion which was much superior to the imbedded material for fine details, it seems certain that all the chromosomes of the prophase are split (fig. 81) and that this condition is maintained through the metaphase. Slight differences in fixation or staining would tend to obliterate these fine separations. A slightly oblique view of the chromosome would also tend to obscure the split.

In recent years the point of spindle fibre attachment has been found important. It is difficult in the material I have of the pig to directly determine this point as the spindles are visible in but very few instances. Indirectly it is possible to obtain some evidence as to the point of fibre attachment from the shape assumed by the chromosomes in anaphase as the form at this time would be dependent upon the point of the chromosome at which the pull was applied. In other words, if the spindle fibre is attached to the end of the chromosome or is telomitic (Carothers '17) the daughter chromosomes pass to the poles as rods whereas if the attachment is along the body of the chromosome or is atelomitic then upon separation the daughters approach the poles as V's with equal or unequal arms depending upon whether the fibre is joined to the exact middle or to one side of the middle of the chromosome. Figure 16 shows rods and V's which have already reached the poles. Figures 84, 87 and 88 show rods V's with equal and unequal arms approaching the poles. From the material studied it may be said that the fibre attachment is in some instances telomitic and in others atelomitic. More chromosomes apparently have terminal fibre attachment than subterminal fibre attachments. I cannot present complete evidence as to the constancy of the point of fibre attachment in the pig but the similar form of certain recognizable chromosomes (the longer ones) in various cells would seem to indicate a permanency of the location of fibre attachment.

A giant cell

Figure 14 is a drawing of the chromosomes of a giant cell found among the spermatogonia. The chromosomes at the right hand end, as the cell has been placed on the plate, are somewhat crowded and indistinct, but there are at least as many chromosomes as are figured. The number is seventy-four, which is just six less than double the normal spermatogonial number. Since the chromosomes do not appear smaller than those in the normal cells and the chromosome number is nearly double that usually found, it is believed that this cell has arisen, either through the fusion of two spermatogonia or because separation of the daughter cells failed to take place after the chromosomes had divided.

The reduced number of chromosomes

Although no particular study has been made of the spermatocyte chromosomes, I have included two very clear figures at the end of plate 2. Both of these show twenty chromosomes. Those familiar with the characteristic shape of the first spermatocyte chromosomes, as found in the grasshoppers, some of the bugs and other forms, will recognize similar forms here. These forms were not shown in the previous study of the spermatogenesis of the pig (Wodsedalek, '13).

THE SOMATIC CHROMOSOMES

The present report must necessarily be preliminary in nature and intended rather to point out certain conditions that exist, than to offer a final solution of the problem. With this in mind, two embryos were selected for the study of the chromosome conditions in the body—one embryo of eight, the other of fifteen millimeters. The smaller embryo was fixed entire, while the larger one was teased. The teasing was not sufficient to interfere greatly with the recognition of the various tissues. The fixation in both was excellent, as may be judged from the drawings, and was fully as good as was obtained in the germ cells. Only those cells were selected for drawing which were clearly uncut and in which the chromosomes were well separated and distinct. Small cells possessing a large number of chromosomes, may, even in the most perfectly preserved material, occasionally present one of two places where the number of chromosomes involved is uncertain. This will be spoken of later.

The chromosome number

It was soon found that the somatic chromosome number was not constant. The variation ranged from forty to fifty-seven, and one cell was found in which at least seventy-four chromatin bodies were counted. Ninety-one cells were counted. (See page 167.) Drawings of the various types of cells found in the embryo are given in figures 23 to 88. Although, at first, the greatest variation was thought to be confined to the blood, as more tissues were studied, it was found that the blood was not an exception to the rule and that a similar range of numbers was present in each tissue in which a number of active cells were found. The following table gives in detail the distribution of the variations in the thirteen tissues studied.

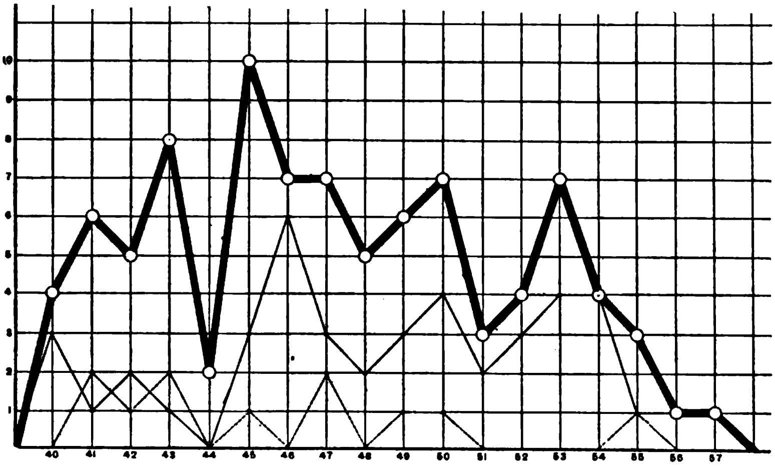
Table of chromosome numbers in the various tissues studied

The figures at the top of each column refer to the number of chromosomes and the figures in the columns indicate the number of examples found

TISSUE	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	74	NUMBER OF CELLS
Blood		2	1	2		1		2		1	1					1			1	12
Brain	3	1	2	1		3	6	3	2	3	4	2	3	4	4	1	1	1		44
Connective			1	3		2				1	1			1						9
Amnion	1	1							1		1									4
Myotome		1	1	1																3
Heart				1																1
Diaphragm					1															1
Wolffian body			1			1			1											3
Lung																1				1
Umbilicus							1	1												2
Limb buds						1			1	1	1		1							5
Optic cup														1						1
Liver						2		1				1		1						5
Number of cases	4	5	6	8	1	10	7	7	5	6	8	3	4	7	4	3	1	1	1	91

Text figure 1 illustrates graphically some of the conditions given in the above table. On this chart no one type or group of cells appears to be predominant. It seems very probable that when a larger number of cells are studied, this curve will 'straighten' out, showing approximately the same number of cells falling in the various classes.

Had the variation in number been but one or two it might have been attributed to errors in observation and drawing because of the many chromosomes in the cells. But the numbers ranged from forty to fifty-seven, which excludes the possibility of error from this source. An error of one or two chromosomes might well be made but, considering the excellence of the preparations and the care taken in checking the drawings, not one of seventeen. In recording the chromosomes my constant effort was to underestimate rather than to overestimate the number. It was frequently difficult to determine whether the chromatin body under examination was really single or composed of two parts. In all such cases, except where I was finally able to determine definitely, I have counted the doubtful element as one rather than two. This difficulty was encountered, particularly in cells possessing fifty or more chromosomes and, in many instances, cells which I have placed in, for example, the fifty chromosome class may well have fifty-one, fifty-two or fifty-three chromosomes. There are certainly, however, at least as many chromosomes in a cell as are indicated by my numbers and my drawings.



Text fig. 1 A frequency curve (heavy band) showing the group distribution of cells possessing various numbers of chromosomes as found in ninety-two somatic cells of the pig. The thin solid line represents the range and distribution of variation for cells of the brain and the broken line does the same for the blood. None of these curves appear to have a definite mode. The number of chromosomes are listed on the base line while the frequencies are recorded on the axis of ordinates.

The chromosome number in uncut membranes

Since the main work of this study was completed I have obtained fresh material from Cincinnati. The amnion of embryo pigs (18 to 20 mm.) was prepared as described (page 160) and was found exceedingly favorable for study. The thickness varies but in the portions observed it was seldom more than three cells thick and frequently the membrane seemed to be composed of a single layer. The certainty that the cells were uncut made the material excellent for checking the results obtained on the earlier sectioned embryos. The clearness of the figures in these membranes is evident from figures 81 to 85 and (photomicrographs) 89 to 93 and 95.

Nearly all the cells studied were in prophase lending further support to the idea of a 'cycle of division' (page 157). Twenty-nine cells have been drawn and counted and the range of number is the same as reported for the rest of the soma. The twenty-nine cells fall into the following groups:

	NUMBER OF CHROMOSOMES											TOTAL
	40	41	42	43	44	45	47	49	50	53	58	
Number of cases..................	10	2	4	1	3	3	2	1	1	1	1	29

There are more cells in the forty class than found in the previous material but, as has been indicated, the frequency curve of the first cells studied was without definite mode and it may well be that the frequencies in this case are also governed by chance.

All that has been said on the structure of chromosomes on page 163 applies to the chromosomes of the somatic cells as well as those of the germ cells.

The behavior of the 'extra' chromosomes

The chromosomes of the spermatogonia have been shown to be constant in number, while those of the soma have a rather wide range. Regardless of how this variation has come about it is interesting to know how these 'extra' chromosomes the chromosomes in excess of forty) behave. Are they carried on as a part of the chromatic complex or are they thrown out to degenerate in the cytoplasm? My evidence on this point is not very conclusive, as it is difficult to find anaphase stages oriented in the proper plane and at just the right stage for counting. In one case, however, I was able to make a fairly accurate count of the chromosomes at one pole of an anaphase group. In figure 57 the plate placed toward the upper side of the page shows at least forty-three chromosomes. There are very likely more present, but forty-three chromosomes were readily discernible. At the lower pole only thirty-six chromosomes were distinguishable, but the spindle axis was slightly oblique to a perfect polar view and consequently it was impossible, even to approximate the actual number of chromosomes present. It is evident in one case, at least, that the 'extra' chromosomes behave as do the others and divide regularly. It is rarely that I have found a cell in anaphase with the chromosomes trailing on the spindle after the others had reached the poles as might be expected if these 'extra' bodies were eliminated. Figures 86 and 88 are thought to be two such cells. In figure 86 there is an area surrounding a trailing chromosome that is lighter in color than the cytoplasm and which appears as the beginning of a vesicle. This vesicle might cause the disintegration of this chromosome outside of the nucleus. The evidence for this, however, is very poor. Evidence from another form on the constancy of the complex will be mentioned presently in support of the above statements.

The cause of the variation in number

Since the chromosomes of the male germ cells are constantly forty in number, how has the soma acquired the wide range of number that has been described? One explanation would suggest that they are giant cells, or perhaps what may be termed partial giants, due to the division, but lack of separation of certain chromosomes, as found by Dr. Caroline Holt ('17) in the cast off lining of the intestinal wall in Culex pipiens. This is not likely for several reasons. In the first place the multiple chromosome cells in Culex are degenerating and abnormal, while the cells in the pig which show variation in chromosome number are perfectly normal in appearance and size, and are part of active, healthily growing tissue. When the somatic cells are compared with the giant spermatogonium reproduced as figure 14, it is very obvious that the cells of the soma are much smaller and appear, so far as the diameter of the metaphase plate is concerned, like normal spermatogonial cells. The other possibility is that the chromosomes in the somatic cells have fragmented. This suggested itself to me particularly, as I had discovered that the variation in the number of somatic chromosomes in the evening primrose, Oenothera scintillans, is due to the fragmentation of certain chromosomes (Hance, '18). In the case of Oenothera scintillans I found the chromosomes showing deep constrictions which led to the solution of the problem in this instance. The conditions in O. scintillans will be discussed later. Although I have searched for such constrictions in the chromosomes of the pig, I have never found any of which I could be certain. In several cases chromosomes have been found in which there appeared to be a constriction but this interpretation was always open to considerable doubt. In figure 36, to the left of the number is one of the best cases of a thin area in a chromosome that I have found. One of the chromosomes in figure 85 also shows constrictions. When a large number of chromosomes are concerned, it becomes a matter of considerable difficulty to determine whether a chromosome has been found showing a constriction or whether this dent is really due to the overlapping or approximation of two chromosomes. Although it has been impossible actually to demonstrate the possibility of fragmentation through the discovery of constrictions in the chromosomes, there are, however, indirect methods of proving that this process is going on in the pig. If the chromosomes are breaking up we would expect to find, as in the case of Oenothera scintillans, that those cells showing a high degree of fragmentation would give ocular evidence of the fact by possessing visibly shorter chromosomes. Those cells which possess only a few more than the spermatogonial number of chromosomes should appear like the germ cells, while those having forty-five or more should have chromosomes which are obviously shorter. A comparison of a cell having many chromosomes with others in the same tissue, or in other tissues having the spermatogonial number or very close to it, will show that the chromatin bodies in the many-chromosome cells are in general, shorter. Compare figures 23 to 80 with figures 1 to 20 and the photomicrographs 96, 97 and 98 with 99.

With the ocular evidence in favor of the breaking up of the somatic chromosomes, further evidence indicating that such a process is actually taking place may be offered. That the 'extra' chromosomes divide and behave normally has been described above. They are not as a rule at least, lost from the complex. If the extra chromatin bodies present are not due to additions to the regular nuclear equipment, but rather to the breaking up of the type chromosomes, the total length of all of the chromosomes in any somatic or spermatogonial cell, regardless of the number of separate elements possessed, should be approximately the same. The results of an investigation to determine this point follow.

THE METRIC ANALYSIS OF THE CHROMOSOMES

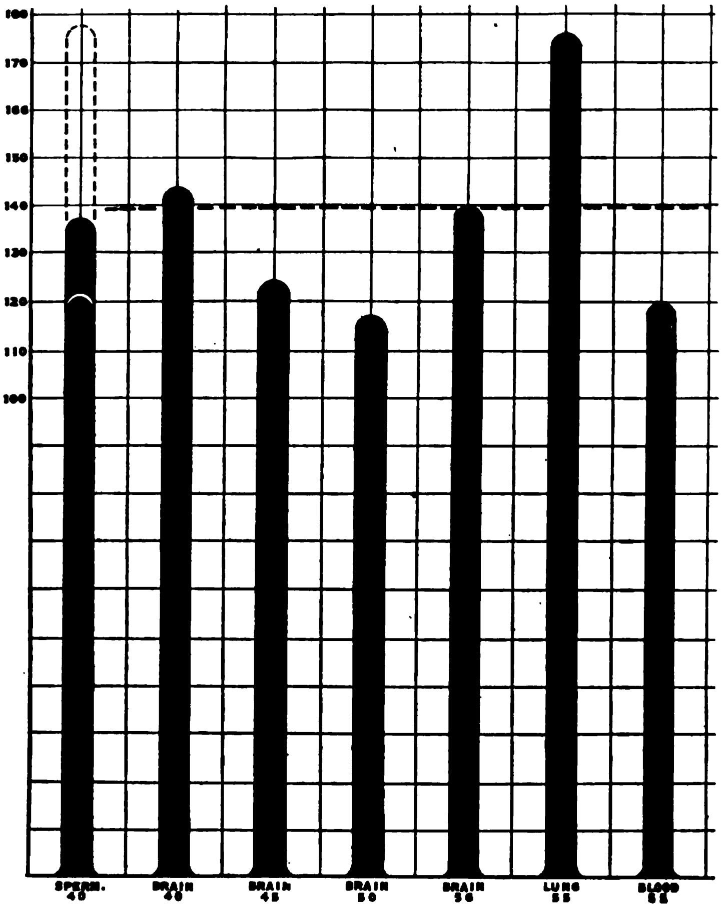
In studying the chromosomes, use has been made of the method first developed in the work on the evening primrose, Oenothera (Hance, '18). Measurements of chromosomes have been made before, but the discovery of the exact inter-pair relationship is, in somatic cells as far as I am aware, new, and should prove of considerable value in studying cytological data (particularly in the determination of the chromosomes involved where irregularities occur). Since these methods were worked out I find that Robertson ('15) has employed the relationship of certain chromosomes expressed in ratios in some of his Orthopteran studies. This will be referred to in the discussion. Sutton ('02) was the first to determine the paired condition of the germinal chromosomes. He pointed out that the pairs differed in length and maintained a definite and constant size from one cell generation to another, and also that the pairs, when arranged according to length formed a practically uniformly graded series. Meek ('12a, '12b and '15) has also made extensive measurements. These methods permit the accurate checking of results and eliminate, to a large degree, the personal element which so frequently enters cytological work. Relationships not optically discernible are brought out, and the application of the method to the work of others enables an investigator to check his work as regards accuracy and interpretations.

Spermatogonia

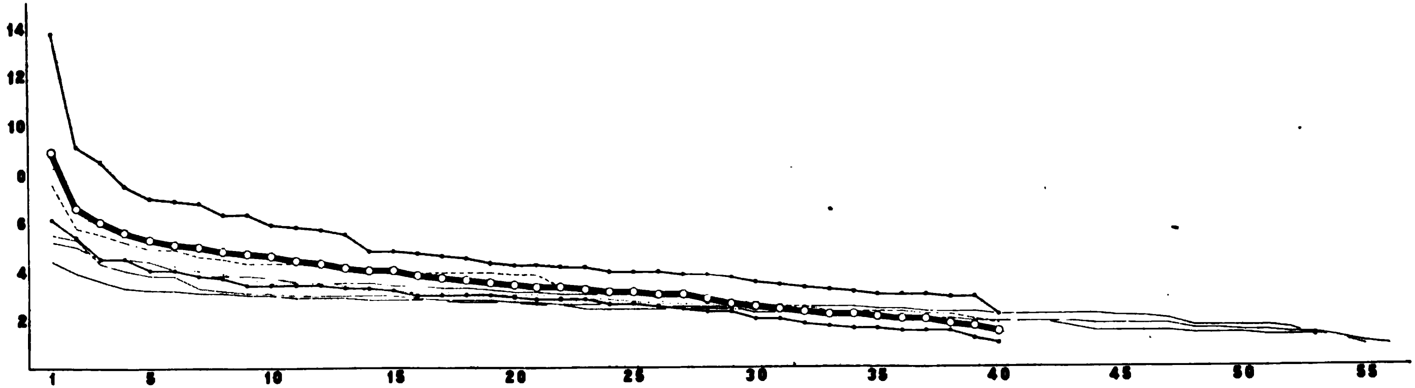
Total length of the chromosomes. The total lengths of the chromosomes in the spermatogonia at 20,400 magnification were found to range from 118.6 centimeters to 177.6 centimeters with an average of 136.9 centimeters. A glance at figures 1 to 20 will show the reason for this wide variation. Cells differ considerably in the size of the chromosomes, although it is apparent from the figures and from the measurements, that each chromosome of a cell suffers equally in any reduction or gains proportionally in an increase of size. I have reported the same conditions, rather more marked, in the somatic cells of the mosquito (Hance '17 a). This may be due to differences in nourishment, or, as in the case of the production of extra contractile vacuoles in paramaecium ('17 c), to a rapidity of division which does not permit the chromosomes time to grow to the limits of their possibilities. A graphic illustration is given in text figure 2 of the minimum, maximum and average length of the spermatogonial chromosomes.

That figure 14 is a giant cell is readily demonstrated when the chromosomes are measured. Although the total lengths of the spermatogonial chromosomes has been found to vary between 118.6 and 177.6 centimeters, the chromosomes of figure 14 measure 235 centimeters, or 58 centimeters longer than the longest set of chromosomes.

Pairs and relation of pairs. When the chromosomes of the spermatogonia are arranged according to length, it is found that they form a series which falls very gradually from the long end of the series to the short end. This may be seen in plate 9, row 1, and it is more clearly shown by the heavy black band in text figure 3. The pairing is not as strikingly obvious as in the case of Oenothera scintillans, but, considering the chances of error in drawing a large number of small chromosomes, I believe that the pairs stand out fairly well. In the table below the average lengths of the spermatogonial chromosomes are listed. Frequently the mates are found near each other in the complex but not always.



Text fig. 2 A graphic illustration of the total lengths of the chromosomes of the spermatogonia and in different body cells which have undergone various degrees of fragmentation. The figures to the left refer to the length in centimeters. The dotted addition to the pillar representing the spermatogonia indicates the greatest length found and the white line shows the minimum for these cells. The heavy dotted line across the diagram represents the average length of the chromosomes of somatic cells. Note that the total lengths of the somatic chromosomes fall within the limits found for the spermatogonia and that the average length for these cells is almost identical to the spermatogonial average.



Text fig. 3 Curves illustrating the chromosome length series. The figures on the axis of ordinates refer to length in centimeters while those along the base line are the numbers of chromosomes arranged according to length. The heavy black band indicates the average length of the individual chromosomes of the spermatogonia. The lighter line interspersed with dots lying above the heavy band indicates the maximum lengths and the similar line below indicates the minimum lengths found in the spermatogonia. Other lines represent somatic chromosomes. These are described in the text.

Table of spermatogonial chromosome lengths, pairs and relations

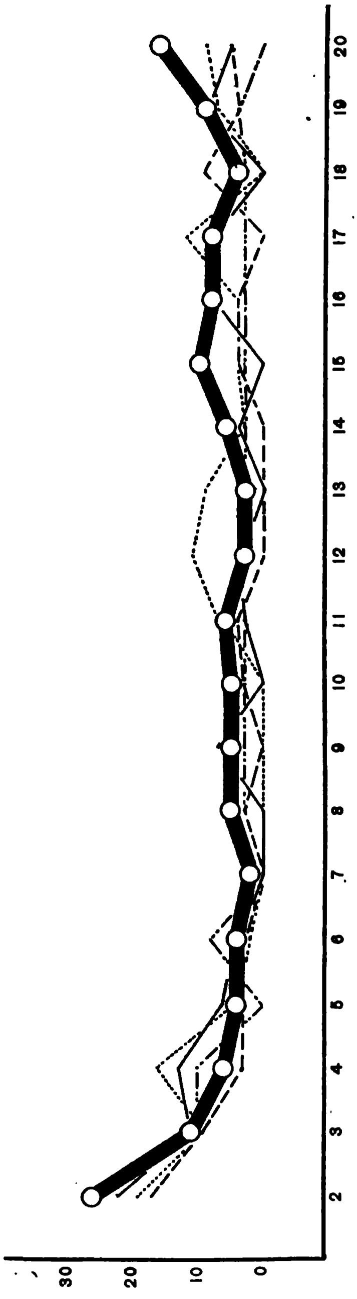
PAIR	CHROMOSOME	LENGTH IN CENTIMETERS	RELATION OF PAIRS IN PER CENT
1	1	8.9	74
	2	6.6	
2	3	6.0	89
	4	5.6	
3	5	5.3	94
	6	5.1	
4	7	5.0	96
	8	4.8	
5	9	4.7	96
	10	4.6	
6	11	4.4	98
	12	4.3	
7	13	4.1	95
	14	4.0	
8	15	4.0	95
	16	3.8	
9	17	3.7	95
	18	3.6	
10	19	3.5	94
	20	3.4	
11	21	3.3	97
	22	3.3	
12	23	3.2	97
	24	3.1	
13	25	3.1	94
	26	3.0	
14	27	3.0	90
	28	2.8	
15	29	2.6	92
	30	2.5	
16	31	2.4	92
	32	2.3	
17	33	2.2	96
	34	2.2	
18	35	2.1	91
	36	2.0	
19	37	2.0	84
	38	1.8	
20	39	1.7	
	40	1.5	

As a constant relationship between chromosome pairs had been found in Oenothera scintillans, it was of interest to determine whether such exists in the pig. That it does is shown in the above table in the column to the right. There the relationship of the pairs is expressed in terms of per cent, i.e., a shorter pair is a certain per cent of the length of the next longer pair, etc. There is a rather large increase in this percentage relationship at either end of the series. These relationships will be more readily understood from text figure 4 when the heavy black band expresses the percentage of difference between pairs of chromosomes in the spermatogonia, drawn from the figures given above. This curve shows a remarkably uniform difference in length between the pairs, except at the two extremities. In cells in which the total length of the chromosomes is short, all of these have been affected equally in the size reduction, as the percentage relationship between the pairs is the same as between the same pairs of the 'longer' cells.

Somatic cells

Total lengths of the chromosomes. The total lengths of the somatic chromosomes in the various tissues is given in the following table. As can be seen, the total lengths of the chromosomes in the somatic cells falls within the limits determined for the spermatogonia. The one exception to this statement—the fifty-chromosome brain cell measuring 117 centimeters—falls below the minimum length found for the spermatogonia by such a small amount that I am not inclined to attach any importance to it. The average length of the various cells given above is 139.3 centimeters, which is exceedingly close to the average found for the germ cells, 136.9 centimeters. The lengths from various cells are compared with those of the spermatogonia in text figure 2. It is evident that, since the total length of the chromosomes in the various classes of cells is approximately the same, the increased number of chromosomes must be due to fragmentation.

Figures 23 to 80 show that any tissue may have metaphase plates that vary as much in total diameter as do those of the



Text fig. 4 This curve illustrates the length difference between chromosome pairs expressed in percent. The numbers to the left refer to per cent and the numbers along the base refer to pairs of chromosomes. The heavy black band indicates the percentage difference between chromosome pairs in the spermatogonial cells and the other lines represent similar relations between the first twenty pairs of chromosomes in highly fragmented cells.

spermatogonia. The measurements given above bear this out. It may be pointed out that, although considerable variation exists between the total lengths of all of the chromosomes in the various cells, this variation is not greater in one tissue than in another and falls within the limits determined for the spermatogonia.

On plate 10 the chromosomes of a number of cells in various degrees of fragmentation are arranged according to size. It is obvious that the somatic chromosomes, particularly at the long end of the series, are shorter than those in the same column in the row devoted to the spermatogonial chromosomes or to the somatic cells possessing forty chromosomes. The conditions illustrated on this plate will be referred to later.

Table of total chromosome lengths in the cells of various tissues and in cells possessing different numbers of chromosomes

TISSUE	NUMBER OF CHROMOSOMES	TOTAL LENGTH OF CHROMOSOMES IN CENTIMETERS
Amnion	41	129.2
Blood	55	129.8
Brain	40	143.9
Brain	45	124.1
Brain	50	117.0
Brain	56	139.9
Limb bud	52	152.6
Liver	53	158.4
Lung	55	176.1
Myotome	43	
Average		139.3

The length of the chromosome fragments. In my studies on Oenothera scintillans I considered all the chromosomes which, when arranged according to length, fell below the shortest chromosome in the normal diploid series, to be portions or fragments of longer chromosomes and I found them to be remarkably alike in length. When the chromosomes on plate 9, to the right of chromosome 40, are studied, it is evident that they, too, vary but little. By actual measurements they average 17 millimeters, with one extreme measuring 9 millimeters and the other 25 millimeters. The majority approach very near the average length. Recalling that the longest chromosomes of the series average 8.9 centimeters, it seems justifiable to consider the chromosomes below chromosome 40 in the length series to be very nearly of even size in the majority of cases.

Discussion of the results obtained by measuring the chromosomes

The conditions in Oenothera scintillans. In order that the metrical studies of the chromosomes of the pig may be better understood a synopsis of similar work on Oenothera scintillans (Hance '18) is given below. As the chromosomes in this Oenothera were much less numerous than in the pig, more clear cut and definite results were obtained, and they aided greatly in the interpretation of the observations on the latter.

1. In material collected from three generations of Oenothera scintillans the somatic chromosome number varies from 15 to 21. This variation is not correlated with any particular tissue, but exists throughout the plant.
2. The type or fundamental number of chromosomes is 15.
3. The 'extra' chromosomes divide regularly.
4. Each pair of the type (15-chromosome) group differs from the next pair in the series by 9 per cent, or a shorter pair is 91 per cent the length of the next longer pair. This is apparently a constant relation.
5. The 'extra' chromosomes have been shown to be fragments of the type chromosomes, and the total length of the chromosomes in the various classes of cells has been found to be practically identical. The fragments are very nearly of even length.
6. With the aid of the paired conditions of the chromosomes and of the relation between the pairs, it has been found possible theoretically to reunite the fragments with the chromosomes from which they have separated.
7. Fragmentation in the cells studied, has been confined largely, but not exclusively, to the longer chromosomes.
8. Fragmentation does not occur in the germ line.

The conditions in the pig. With the increase in the number of the chromosomes the difficulties of analysis are increased. It is consequently necessary to know how far we may trust the accuracy of the present work before it is possible to draw conclusions from the results. If the majority of the results obtained in the present study can be shown to be safely comparable to those obtained on O. scintillans, it will be justifiable to assume that the conclusions reached for the earlier and more favorable form will be applicable to the pig.

A very interesting relation exists as regards the degree of fragmentation in the two forms. Considering only the cells in which the highest number of chromosomes is found in both the Oenothera (21 chromosomes) and the pig (57 chromosomes) in comparison to the type or fundamental numbers (15 and 40 respectively), it becomes evident that the evening primrose has suffered a fragmentation of 40 per cent of its chromosomes and the pig of 42.5 per cent. While future work may show that this percentage of fragmentation may mean nothing and is merely a coincidence, for the present it is suggestive.

To review briefly the results obtained in the study of the chromosomes of the pig:

1. The spermatogonial number is 40.
2. The somatic number as determined in the present study ranges from 40 to 58.
3. The 'extra' chromosomes divide as do the type chromosomes.
4. A difference averaging 12 per cent has been found to exist between spermatogonial pairs. If the relationships of the length of the pairs on either end of the series be omitted the average per cent is but five.
5. The total length of the chromosomes of any somatic cell, regardless of the number of chromosomes or of the tissues of which it is a part, falls within the length limits found for the spermatogonial chromosomes.
6. The 'extra' chromosomes, for reasons set forth above, must be fragments of the normal chromosomes.
7. The fragments are very nearly of even length.

8. Fragmentation does not affect the germinal chromosomes.

A comparison of the conditions in the two forms. When the summary of the observations on the pig is compared with the preceding one on Oenothera scintillans it is plain that, with one or two exceptions, the words pig and Oenothera could be interchanged without affecting the truth of the statements in the slightest. The relationship between the pairs differs slightly, but is, however, fairly constant in both species. The chief discovery in the case of the Oenothera is that it is possible to reunite the fragmented chromosomes and thus determine, with a fair degree of accuracy, which chromosomes have broken up. Is this method of procedure applicable to the chromosomes of the pig? Can the chromosomes which have fragmented be located? The chance of even approximate accuracy in attempting to reunite the fragmented chromosomes of the pig is so slight that it would be unwise to base any conclusions on the results. This is true for the following reasons: 1) The large number of chromosomes and the consequent and very probable slight inaccuracies in drawing are prohibitive of the success of such a study. 2) The very slight differences, in the majority of instances, between the chromosome pairs present no definite criterion on which to base judgment as to which chromosomes are out of place in the series and consequently with which fragments they should be united.

It is possible, in a more direct way, however, to determine which chromosomes of the series are breaking up. The method is not so completely satisfactory as the one used with the evening primrose, but serves to show, in a general way and with a considerable degree of accuracy, which end of the chromosome series is breaking up. In plate 9 it will be recognized at once that, in general, in those somatic cells (row 3 to 12) possessing a large number of chromosomes, the chromosomes at the beginning of the series are shorter than the chromosomes of the spermatogonium (row 1) and of the unfragmented brain cell (row 2) in the same columns. There are exceptions to this which tend to show that, in breaking up, the chromosomes may not follow a definite plan beginning with the long chromosomes and proceeding down the series. In row 6 a 41-chromosome cell is represented. Here the first two chromosomes are very clearly entire, judging their lengths by those of the first two chromosomes of row 1. It appears very probable that either chromosome 3 or 4 has been the one to lose the portion represented by chromosome 41. In row 11 the first two chromosomes are long, but so, also, are the other chromosomes in this cell, and when considered from this point of view these chromosomes are relatively very nearly as short as the first pair in row 10. The chromosomes in row 11 measure 176.1 cm., while the total length of those in row 16 is 153.6 cm. Row 11 is therefore 8.8 per cent longer than row 10. If the total lengths of the chromosomes vary by 8.8 per cent then, according to the statements made above that in reduction (for whatever reason) each chromosome suffers proportionately, any corresponding pairs from either of the cells should differ by very nearly the same amount. It is, of course, not expected that exact results may be obtained in all cases for reasons pointed out earlier. Consider for example the pairs composed of chromosomes 1 and 2 in rows 10 and 11. These chromosomes average 5.1 cm. in length in row 10 and 6.7 cm. in row 11. and consequently differ by 7.6 per cent. In this instance there is a discrepancy of 1.2 per cent. When the next pair in each cell is compared it is found that the difference is 8.8 per cent exactly the same as the variation between the total lengths. This is sufficient, I think, to indicate that the difference in total length is due to a proportional reduction of the chromosomes of row 10 and that the corresponding pairs in each of the rows are comparable. Furthermore it is evident that the longer chromosomes in both rows have in all probability lost a portion of their length since they are shorter than the corresponding 'typical' pairs.

The fact that it is the members of the long end of the chromosome series which are breaking down becomes more evident in text figure 3. For the sake of clearness the graphs for only three greatly fragmented cells have been drawn. These are represented in light unbroken lines. Note that in all three cases the curves start out below the curves representing the minimum lengths found for the spermatogonial chromosomes. One cell enters within the spermatogonial minimum curve at chromosome 3 and continues, either on it or very close to it, for a considerable distance. This, however, is a 'longer' cell than the other two and would be expected to rise above the type minimum more quickly than the others. *It does not, however, rise noticeably above the minimum curve at the long end of the series*, notwithstanding its extra total length. The other two curves do not rise above the minimum curve until chromosome 27 is reached. These three cases, which are typical for others, most certainly show that the chromosomes at the long end of the series have been reduced considerably in length. This becomes especially clear when the broken lines in text figure 3 are followed and contrasted with the results mentioned above. These curves represent one cell with unfragmented chromosomes and another in which there is a single fragment. The curves lie within the extremes of the spermatogonial lengths for the full distance, and the curve representing the chromosomes of the unfragmented cell coincides with the spermatogonial average curve for a great part of its length.

Hence it seems entirely justifiable to claim that, although the exact chromosomes which have broken up cannot be ascertained, as was done in the case of Oenothera scintillans, the same general conditions hold true, namely, that the chromosomes of the long end of the series have fragmented. This, of course, does not exclude the possibility that some of the shorter chromosomes have broken up. Since this was discovered in Oenothera scintillans, and since the other conditions in both forms are so comparable, it seems probable that the fragmenting of some of the shorter chromosomes may occur in the pig. Neither has it been possible to show that a chromosome has not lost more than one piece. For various reasons, however, I am, at present, inclined to think that a chromosome does not lose more than one piece or, if it does, that the condition is rare.

The point of fragmentation. That the fragments should be so nearly of even length in the two forms studied is very suggestive. and would seem to indicate that one or two chromomeres have broken off and that the loss is limited to that amount of chromatin. Since the fragments are fairly uniform in length, the chromosomes must be reduced by more or less equivalent amounts, and consequently we should not expect to find the percentage relation between the pairs showing any marked variation from that found between the spermatogonial pairs. This is shown graphically in text figure 4 where the percentage relations of the first twenty pairs of four highly fragmented cells are plotted. As can be seen, the curves vary but slightly from the spermatogonial curve.

It has been pointed out that the long chromosomes are the chief ones involved in fragmentation. A study of the longer chromosomes Plate 10, rows 1 and 2 (unfragmented cells) reveals that several are J-shaped or formed as an unequal armed V. Assuming that the point of spindle fiber attachment is located at the apex of the J (page 163) the possibility that fragmentation has occurred at this point immediately suggests itself since the short arm of the chromosome is in many cases approximately the length of the chromosomes (the fragments) lying above 40 in the fragmented series. It will be noted on Plate 10 that there are several J-shaped chromosomes in those rows representing cells in which little or no fragmentation has occurred. With an increasing degree of fragmentation the number of curved chromosomes becomes less and less. In cells with high numbers of chromosomes those lying in the same columns as the curved chromosomes of rows 1 and 2 are generally rod-shaped. While this is but circumstantial evidence it offers a logical and interesting explanation of the process as occurring in these cells. It is, of course, but a tentative theory which may be proved or overthrown by further work.

DISCUSSION

The work of other mammalian cytologists

The amount of published cytological work on the mammals is neither large nor reliable, when compared to that which has been done on other forms as, for instance, the grasshoppers and the bugs. Since the present paper deals with the diploid number alone, I will consider only that part of the reported mammalian investigation. It is rather interesting to note the elaborate descriptions of the resting and prophase conditions and the brief consideration and few figures of the chromosomes themselves. The inference is rather obvious, and it is partly to counteract the impression that sufficiently clear, and easily studied chromosome complexes are scarce in mammalian material, that I have presented the large number of figures at the close of this paper.

The investigation of the most importance to the present study is Wodsedalek's work on the pig ('13). He has reported 18 chromosomes in the spermatogonia and 20 in the ovary. He also found two classes of embryos, one with 18 and the other with 20 chromosomes. He figured two X-chromosomes. The discrepancy between Wodsedalek's count of 18 chromosomes and my own of 40 chromosomes in the same type of tissue is rather large, and I feel sure, has been due largely to the difference in the methods of preparing the material. In a paper on "The fixation of mammalian chromosomes" ('17 b) I have described the result of fixing mammalian tissue with various fluids, and also the effect of fixing stale tissue (which, indeed, may not have been removed from the body for more than 15 to 20 minutes). Wodsedalek's figures are practically identical with those I have found in either poorly fixed tissue or in tissue that was dead when preserved. The chromosomes are clumped and undecipherable, as is evident in the one spermatogonial and three embryonic diploid cells figured in Wodsedalek's paper. A very interesting condition seems generally to occur when chromosomes clump together as the result of poor fixation. The number is reduced to approximately one half through the fusion of chromosomes lying along side of each other—probably mates. In the case under discussion, Wodsedalek's count is two less than one half of the count I have made. In this condition, such chromosomes as can be distinguished at all, are at least double the width of those found in properly fixed material. It is very evident that such poorly preserved tissue should never be accepted as available for study, since any conclusions based on such material are not only open to criticism, but are very probably wrong. A form possessing so large a number of chromosomes as the pig enclosed in so small a compass must, indeed, be prepared with the greatest care if results of any value are to be obtained.

It is possible to show more conclusively that the diploid chromosomes of Wodsedalek's pig possess the same amount of chromatin as I have described for the number of individuals studied. If a fusion between adjacent chromosomes, or perhaps between pairs, has occurred, the total length of the chromosomes in Wodsedalek's figures should be about one half the length I have found. Furthermore, the chromosomes (provided the magnification was the same) should average about twice the width of those figured in this paper. It seems probable that, however much the widths may have been effected through poor fixation, the lengths may not have been greatly disturbed, or, if they were shrunken, the shrinkage would affect all alike. Consequently I believe that the measuring of these chromosomes may be relied upon to give fairly accurate results. The chromosomes of the four figures in his paper were copied and enlarged six times over the published size as carefully as possible with the following results.

The length of the chromosomes in the figures of the diploid complexes published in Wodsedalek's article on the spermatogenesis of the pig ('13)

TISSUE	FIGURE NUMBER	NUMBER OF CHROMOSOMES	TOTAL LENGTH × 6 IN CENTIMETERS
Spermatogonium	18	18	56.5
Oogonium	60	20	49.2
Mesonephros	59	18	44.3
Mesonephros	62	20	47.6
Average			49.6 or approximately 50 cm.

The magnification used by Wodsedalek was about one third less than that I have employed. Consequently one third must be added to 50 cm. to bring it up to a magnification corresponding to that used in the present work—50 + 16 = 66 cm.

The total average length of the spermatogonial chromosomes in my material—136.9 cm.

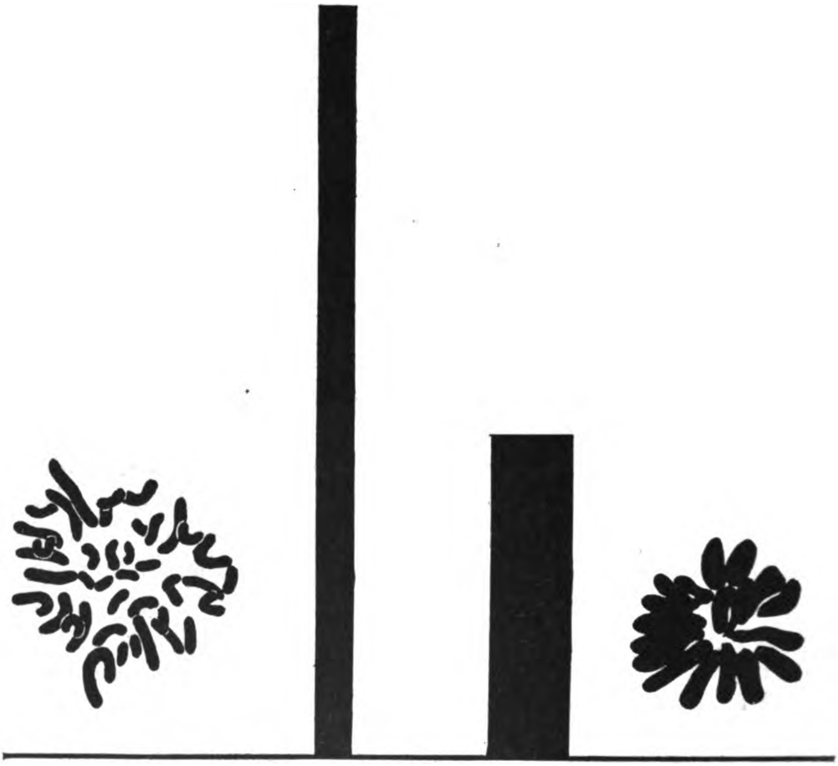
The total average length of the dipoid chromosomes in Wodsedalek's material—66.0 cm.

The length of the latter chromosomes is 48 per cent that of the upper lot, or very nearly one half—66 cm. × 2 = 132 cm. This is so near the average length of the spermatogonial chromosomes found in my work as to be practically identical. In the same way the widths may be determined. I was hampered again by being unable from the figures to determine certainly the limits of the chromosomes, but I think that any error that may have been introduced has been largely checked by averaging the results.

Average chromosome width—12 mm.; + $\frac{1}{3}$ magnification—16 mm. Average chromosome width in my material—7 mm.

The first set is a trifle more than double the width of the latter, not a sufficiently great difference, however, to be significant, considering the chances of error. Text figure 5 illustrates the above results graphically. At the right is the figure of the spermatogonial chromosomes in Wodsedalek's paper, and a column drawn to scale from the above calculations illustrating the average total length and average width of the chromosomes. To the left are corresponding figures representing the present work. This column is obviously a little over twice as high and one half as wide as the column to the right. The area of the left hand column (as reproduced) is 468.5 square mm.; of the right hand column, 441 square mm. It is safe to conclude therefore that both Wodsedalek and myself have been dealing with the same amount of chromatin, and the difference lies wholly in the difference of the preservation of the chromosomes; those in the former study being clumped and undecipherable, while those reported here are well separated.

Though I have little, direct proof to offer at present, I am inclined to believe that other features of the chromosome history reported in the earlier paper, particularly as regards the number of chromosomes in the first and second spermatocytes, and the secondary reduction phenomena in the second spermatocytes, were influenced by the same conditions which affected the number of spermatogonial chromosomes. The first spermatocyte cells that I have counted have had twenty chromosomes, and the secondary spermatocytes—although I have not found



Text fig. 5 A diagrammatic comparison of the amounts of chromatin in pig spermatogonia studied by Wodsedalek and myself. The metaphase plate to the left shows forty chromosomes (× 3400), the column next to it represents the total length of the chromosomes and average width or diameter of the spermatogonial cells. The column and chromosome group (showing presumably eighteen chromosomes (× 3495)) to the right illustrates the same features for the spermatogonial figure published by Wodsedalek. It is evident that the pillar to the left is about twice as high and one half as wide as the one to the left, both consequently possessing the same area. See text.

any in which an accurate count could be made—have shown no tendency to a secondary or pseudo-reduction. Neither have I, in the present study, seen any bodies comparable to the X-chromosomes shown in Wodsedalek's figures. In poorly fixed material obtained some years ago I have seen similar bodies. This problem I hope to take up shortly.

Other cytological work carried out on mammals includes studies on man by Flemming ('98), Farmer and Moore ('05), Moore and Arnold ('06), Duesberg ('06), Guyer ('10), Montgomery ('12), Winiwarter ('12), Jordan ('14) and Wieman ('13 and '17). The diploid number as given by these workers varies from 22 to 47 or 48. The rabbit is claimed to have chromosomes ranging in number from 22 to 80. This form has been studied by Flemming ('98), Winiwarter ('00), (who believes that the somatic number ranges from 42 to 80), Barratt ('07) and Bachhuber ('16), Miss Stevens, in 1911, reported 56 chromosomes for the guinea pig. Vom Rath, in 1894, found the somatic chromosomes in the dog to range from 8 to 64, and pointed out that the various numbers were always multiples of eight. Winiwarter and Sainmont ('09) described 36 chromosomes for the sex and somatic cells of the cat. In the opossum Jordan ('11), found 17 chromosomes in both germ and somatic tissue. Schoenfeld's work on the bull ('07) is largely a study of the prophase behavior, and the number of chromosomes is not given. Wodsedalek found 37 chromosomes in the horse ('14) and 51 chromosomes in the mule ('16). Yocum ('17) has reported 20 chromosomes as the reduced number in the mouse. He has not counted any spermatogonial complexes. His drawings show the chromosomes to be much better separated than is the case in the majority of published mammalian studies.

If we may judge the accuracy of the count from the character of the published figures accompanying the above papers, I believe that we are justified in accepting but few of the writers' conclusions. The chromosomes are massed together, as shown in the case of Wodsedalek's work on the pig, and it seems very likely that fusion of the chromosomes has occurred in the various forms mentioned above, as has been shown to have occurred in the previous work on the pig. In a study of the tissues from seven mammals in connection with work on "The fixation of mammalian chromosomes" (Hance, '17 b) the chromosomes of none of the forms studied, when killed and treated in the proper way, were clumped together or variable in width. They did not look like amorphous lumps of stainable material which is the appearance of many of the mammalian chromosomes in published figures. Furthermore, the number was always high, in all cases about 40 or more. With a large amount of material from so many mammals, I think that I have a fair basis for the criticisms I have made of the mammalian cytological studies up to date. Winiwarter's work stands out above that of other workers, if we may judge the excellence of his fixation from the clearness of his published figures. The diploid chromosomes he figures for the cat are very comparable, in form and number, to those I have found in this animal. I have already suggested ('17 b) that when a study of human cytology is repeated on properly preserved and freshly killed tissue, the number of chromosomes will be nearer that reported by Winiwarter than by other investigators. The variations in the somatic chromosome number which he has found in the rabbit are very suggestive from the point of view of the present work and will be considered later. Miss Steven's figures of the spermatogonia of the guinea pig ('11), although she found but few clear cells, appear to justify her count of 56. Wieman's prophase figures, in his paper on the chromosomes found in a human embryo appear considerably better than do his later figures on the spermatocytes ('13 and '17). Wodsedalek's figures of the chromosomes of the mule ('16) are somewhat better, as regards separation, than those in his papers on the horse and the pig, but all leave much to be desired in the way of regularity of chromosome outline and differentiation of chromosomes.

Reported cases of fragmentation

The most familiar case of variation in somatic chromosome number is that of Ascaris megalocephala reported by Boveri. Here the fertilized egg starts with two chromosomes, and the cell, which may be considered as the anlage of the future germ cells, retains the two chromosomes intact, while the chromosomes of the cells forming the body tissues break up into as many as sixty fragments. This fragmentation is said to be accompanied by a chromatin diminution. If this be true, the quantity of the chromatin in the cells of the soma is probably not the same as it is in the germ line, contrary to what I have found in the pig and in Oenothers scintillans. A metrical study of the chromosome behavior in Ascaris would be of considerable value. Nachtsheim ('13) found 32 diploid chromosomes in the bee, while Petrunkewitsch ('13) found 64 in the blastoderm of fertilized eggs. Hoy ('16) points out that "though the reports of several investigators tend to show that the number of chromosomes varies in the bee, the number is always 8 or a multiple of this." (Nachtsheim found that the number in the oogonia was 16 and in the oocytes 8. He considers that oogonial chromosomes are double, thus accounting for the 32 chromosomes found in the soma.) This condition is from one point of view, comparable to that found by Dr. Caroline Holt ('17) in the cells of the cast off intestinal epithelium of the mosquito, where the number varied from 6 to 72, always in multiples of three. These are degenerating and consequently pathological cells, and differ in this respect from the normal cells of the bee.

In the Hymenopter, Nematus ribesii, Doncaster ('07) found the diploid and the haploid chromosome number to be 8 and 4 respectively, while he counted 16 chromosomes in the ovarian sheath. He considers that the chromosomes of the germ cells may be compound and consist of a number of smaller units which become separated in the somatic cells.

I am glad to have this opportunity of correcting two regrettable errors that were accidentally inserted in my paper on Culex pipiens ('17) in regard to Seiler's work on Phragmatobia ('14). In one place I coupled his name with Taylor's as having worked on the mosquito, when the other name should have been Lohman, and, in referring to his work on the moth, said that he found the somatic chromosome number to be the same as the diploid number of the germ cells. The diploid number is 56 although 58, 61 and 62 chromosomes have been found in the soma. This has been brought about by the fragmentation of the X and Y chromosomes. It is confined to these bodies, and Seiler points out that the breaking up of the sex chromosomes in somatic nuclei has been observed quite often.

The extra number of chromosomes found in giant cells is not due to the same causes as the above, and they are found in the germ tissue as well as in the soma. I have shown the chromosome relation of a giant cell to the normal cells.

The work of Della Valle ('09) and his conclusions in regard to the variability of chromosomes, is so obviously faulty that I shall not consider them here, particularly since Dr. McClung has discussed his observations and theories at length in a recent paper ('17).

From the above synopses of reported cases of chromosome variations it is evident that the somatic number has been discovered to be different from the spermatogonial number in a few cases and that this is largely due to the breaking up of the major or, what I have termed, the 'type' chromosomes, and in certain cases the same chromosomes are always affected while the others remain intact. If the various workers had measured the chromosomes in the tissues studied we should have more information in regard to the constancy of the chromatin elements.

The metrical study of chromosomes

Not very much work has been done in the way of measuring chromosomes, and some of the studies along this line have not produced results of value. Among these I think we may place the work of Meek ('12 a, '12 b and '15), whose work was exceedingly carefully executed, but whose conclusions had to be retracted eventually as they were too sweeping and based on insufficient data.

Farmer and Digby ('14) found that the "total amount of chromatin substance in the nucleus of each of two types of hybrids known as Primula kewensis is the same. The nuclei of the one form of hybrid contains twice as many chromosomes as the nuclei of the other type, but the increase in number is associated with a corresponding diminution in size."

Meves ('11) found a graded series of chromosomes in the salamander He did not believe that his results showed that pairing exists, but it appears to me that the pairing in his material is as marked as can be expected, considering the possibilities of error through foreshortening and in drawing. This becomes especially evident when the exceedingly long chromosomes of the salamander are recalled.

Robertson ('15) made use of a constant length relation between certain chromosomes to determine the amount of deficiency of chromosomes which had lost a portion of their length, and for the relation of the chromosomes of one type of cells to that of another ('16). The relation of chromosomes in the Orthoptera is evidently as constant as in the case of the two forms I have studied. In a very few measurements of the chromosomes of grasshoppers that I have made, the relationship between pairs is very similar.

I feel certain that many valuable results will be obtained through the measuring of chromosomes and the study of their relation to each other and to the chromosomes of closely related groups. Other applications of these methods have been pointed out in my paper on Oenothera scintillans ('18). In a preliminary survey of the difference in length between the various chromosome pairs of several plants and animals, I have found this relation (between pairs) to be practically constant in any one group and, furthermore, in the few examples thus far examined, it does not vary greatly between widely separated groups. What this means is, of course, impossible to imagine or predict at present. It is exceedingly suggestive, however, of a physical or physiological phenomena which future discoveries may show to be of fundamental importance.

Chromosomes in development

At the beginning of this paper it was pointed out that an investigation of somatic chromosomes was really a study of the "behavior of chromosomes in development." We have not, I fear, progressed very far toward the solution of the problem, but several points have been developed which are of some interest and may form a basis for future work along these lines. The fact that the chromosomes may fragment in the soma has been shown to be meaningless, so far as disturbing the quantity of chromatin is concerned, in both the pig and Oenothera scintillans. A definite and apparently constant relation between the chromosome pairs has been discovered which will be of great value in analysing unusual chromosome conditions, such as fragmentation, multiple chromosomes, and the total loss of parts of chromosomes.

Contrasted to the breaking up of chromosomes we have the fusion of certain elements, as described by McClung ('17), Robertson ('16) and Woolsey ('15) and the loss of a portion of certain chromosomes; Carothers ('13 and '17), Robertson ('15) and Wenrich ('16). In these cases the chromosome phenomena link up closely with the taxonomic peculiarities. With the exception of those cases in which certain chromosomes are deficient, the total length of the chromosomes of the cells where these other phenomena have occurred, is, as expected, the same as in the cases where the chromosomes have not fused. The fusion has come about simply as an end to end union. What these phenomena may mean or how or why they have occurred is as much of a riddle as is the breaking up of chromosomes.

A great mass of unquestionable work on plants and animals has demonstrated that the somatic number is the same as the diploid number of the germ cells. To mention but a few—the studies on the squash bug (Morril, '13), Hoy ('16), those on many of the Oenotheras (Gates '15), the work on the mosquito and the Drosophila (Metz, '14 and '16, Whiting, '17 and Hance, '17) have all irrefutably shown that the two types of cells are alike in chromosome numbers.

What, then, does the fact mean that, on the one hand, we find the somatic chromosomes breaking up, while, on the other, they are constant in form and number? In the case of Oenothera scintillans I have pointed out that this is the most unstable of all the 'mutants' of the Oenothera series and never breeds true.

It may be that this breaking up of the chromosomes is correlated with the genetic impurity of the plant. The mosquito is without doubt a 'pure' form, and its somatic chromosomes are constant in number. It is difficult to see how Ascaris can be anything but a homozygous form, and yet the somatic chromosomes are highly fragmented. The pig is probably not pure, considering the length of time it has been domesticated, and its somatic chromosomes show a variation in number. The evidence would seem to be rather evenly balanced at present and no conclusions can be safely drawn.

I think, however, that it may be said with some assurance that the present work, and such previous work as is reliable, has tended to show that the chromosome equipment is the same in the various cells of the body, and when fragmentation occurs, it has not been found to affect one tissue more severely than another. In other words, no tissue specificity of the chromosomes has been found.

The fact that the chromosomes of the majority of somatic cells are more or less fragmented would lend some support to the theory that a germ line or cycle exists, the cells composing which are set off at an early period in the history of the individual. While it is not impossible (from the evidence of the present work) that the germ cells may come from already specialized tissues (Hargitt, '17), it seems rather improbable that cells in which the chromosomes, in all probability, have broken up should be able to reform the typical germinal complex. In view of the present work it seems very likely that the anlagen of the future reproductive cells are set off or guarded from the processes which are taking place in the soma, early in the life history of the animal. Otherwise, since, in the majority of somatic cells studied, the chromosomes break up to a greater or lesser degree, we should have to postulate, either that the chromosomes have the power to recombine as stated above, or that certain cells of some tissue do not undergo the changes common to the rest of the soma. The latter supposition is essentially the germ cycle theory.

The individuality of the chromosomes

It is not my purpose to enter into a full discussion of the theory of the individuality of chromosomes, as Dr. McClung has thoroughly reviewed this subject in a recent publication ('17). I wish merely to add the proof of the present work to that which has already been done. What follows may have more weight when it is said that I began these investigations with the purpose of disproving the hypothesis and was forced into the ranks of the 'believers' by the unquestionable results obtained.

It has been shown that in the somatic cells the chromosomes which are unfragmented agree in length, chromosome for chromosome, with those of the spermatogonia, and the relation between the pairs is the same. When the chromosomes break up, the fragments behave as entire chromosomes at division, and the total length of all the chromosomes of the cell is the same as in the unfragmented cells and the spermatogonia. The quantity of chromatin is undisturbed. What I have said in regard to the chromosome situation in Oenothera scintillans, in respect to the individuality hypothesis, may well be said for the similar conditions in the pig.

> Since no chromatin appears to be lost in the processes that are going on in O. scintillans and as the fragments divide as regularly as they did before they were separated it would seem that at least the spirit of the theory of the individuality of chromosomes was maintained. If the theory of the individuality of chromosomes can only recognize a strict morphological continuity then the chromosomes in scintillans lose their individuality through breaking up. If the theory is broader in its scope and admits an individuality not only of whole chromosomes but of the chromatin or chromomeres then the scintillans situation falls within its limits. It is the latter interpretation of the theory that I believe cytologists hold or are tending toward today.

SUMMARY

Technique

1. An improved preservation of mammalian tissue has been obtained through the use of Flemming's (strong and weak) solution, to which has been added a little urea and which is

used at a temperature of four degrees Centigrade. The pieces of tissue fixed must be very small and absolutely fresh (p. 158).

Observations

2. The number of spermatogonial chromosomes is 40 (p. 162 figs. 1 to 20).
3. The reduced or first spermatocyte number of chromosomes is 20 (p. 164, figs. 21 and 22).
4. The somatic chromosomes range in number from 40 to 58—one cell with 74 chromatin bodies has been found (p. 165, figs. 22 to 80).
5. The 'extra' chromosomes divide as do the type chromosomes (p. 168, fig. 57).
6. The variation in number is caused by the fragmentation of certain chromosomes (p. 169).

Analysis of the chromosome conditions

7. The total length of the chromosomes (× 20400) of the spermatogonia varies from 118.6 cm. to 177.6 cm.; average, 136.9 cm. (p. 172).
8. When the chromosomes are arranged side by side according to length, they form a series which falls very gradually from the long end to the short end of the series (p. 172).
9. In such a series the chromosomes are found to lie in pairs (p. 172).
10. A fairly constant difference in length exists between the pairs (p. 176).
11. The total length of the chromosomes in the somatic cells (× 20400) ranges from 117 cm. to 176.1 cm., showing these cells to be within the spermatogonial limits (p. 176).
12. No tissue specificity of chromosome behavior has been found (p. 165).
13. The chromosome fragments are very nearly of even length suggesting that the chromosomes lose a portion constantly represented by one or two chromosomes (p. 178).

14. The conditions found in Oenothera scintillans and the pig agree in practically all details (p. 181).

15. The chromosomes at the long end of the series are the ones that are chiefly concerned in fragmentation (p. 183).

16. Fragmentation does not enter the germ line.

Discussion

17. Much of the cytological work that has been done on mammals is believed to be faulty due to improper fixation (p. 184).

18. Previous studies on the pig are reviewed and it has been demonstrated that, although only 18 chromosomes were reported, the actual amount of chromatin present is probably the same as found in this investigation. The chromosomes as reported are fused and clumped together in the earlier work; due to poor fixation (p. 185).

19. The various types of chromosome variation (multiples, deficient chromosomes, the loss of chromosomes and fragmentation) that have been studied recently are briefly discussed (pp. 190, 194 and 232).

20. It is pointed out that although many of these peculiarities are closely linked with taxonomic differences, the cause of these variations is still obscure (p. 194).

21. The additional evidence that the present work affords the theory of the individuality of chromosomes is commented upon (p. 196).

Results of the investigation in brief

The chromosomes of the soma may fragment but the fragments continue as part of the complex. The total amount of chromatin in the somatic cell in which fragmentation has occurred is the same as in the spermatogonia. There is no fragmentation in the germ cells, and the chromosome complex remains unchanged.

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DESCRIPTION OF PLATES

Figures 1 to 88 were drawn with the aid of a camera lucida using a Zeiss 1.5 mm. apochromatic objective and a 12 × compensating ocular. This gave a magnification at table level of 3400 ×. The pencil drawings were then enlarged to twice the original size with an accurate pantograph. They are reproduced two-fifths off at a magnification of 4080 ×. In all cases where chromosomes are believed to have divided the mates are connected with a bar except when the separation appears as only a slight slit.

Figs 1 to 80 have been drawn from material fixed in Flemming's strong solution. See page 217 for complexes found in material preserved in Flemming's weak solution.

PLATE 1

EXPLANATION OF FIGURES

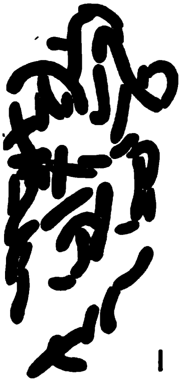
Spermatogonia from three breeds of pigs

FIGURE NUMBER	STAGE	BREED	ANIMAL
1 and 2	Prophase	Jersey red	a
3	Metaphase	Berkshire	f
4 and 5	Metaphase	Berkshire	f
6, 7 and 8	Metaphase	Jersey red	a
9	Late prophase	Jersey red	c
10	Metaphase	Jersey red	a
11 and 12	Metaphase	Poland China	

THE DIPLOID CHROMOSOME COMPLEXES OF THE PIG

ROBERT T. HANCE

PLATE 1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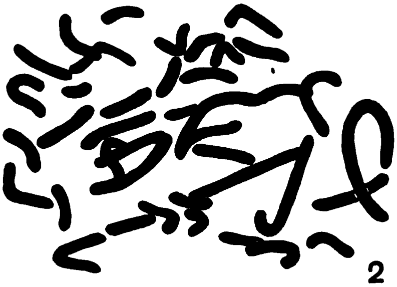
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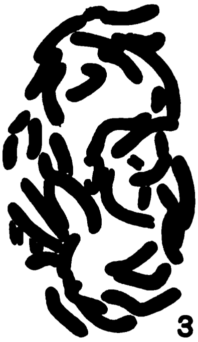
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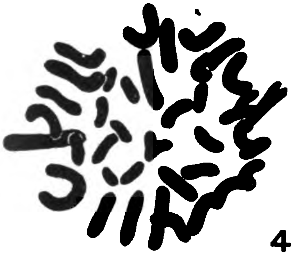
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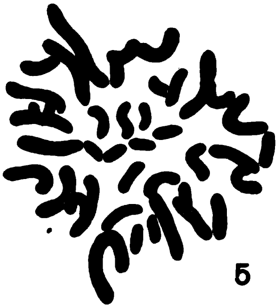
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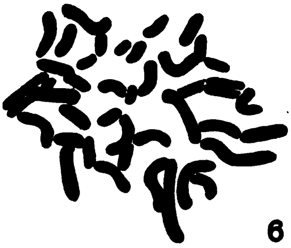
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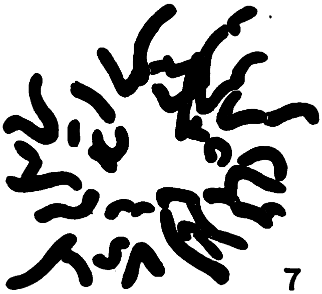
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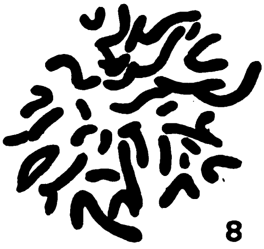
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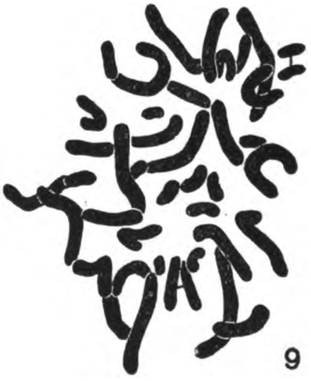
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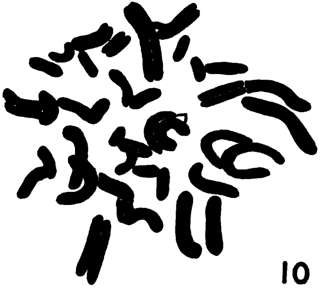
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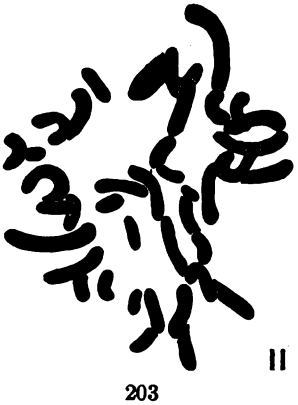
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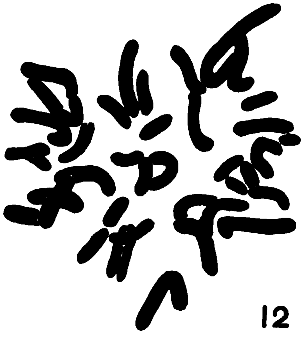
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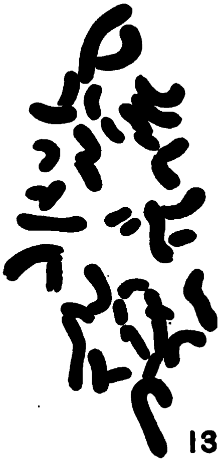
PLATE 2

EXPLANATION OF FIGURES

Spermatogonia and first spermatocytes

FIGURE NUMBER	STAGE	BREED	ANIMAL	NUMBER OF CHROMOSOMES
13	Metaphase	Berkshire	f	40
14	Giant cell spermatogonium	Berkshire	f	74 or more
15	Metaphase	Berkshire	f	40
16	Anaphase	Berkshire	f	40 at pole to the left including chromosome lying between poles. Poles displaced in drawing
17, 18, 19 and 20	Metaphase	Jersey red	a	40
21 and 22	Spermatocyte I. Metaphase	Jersey red	a	20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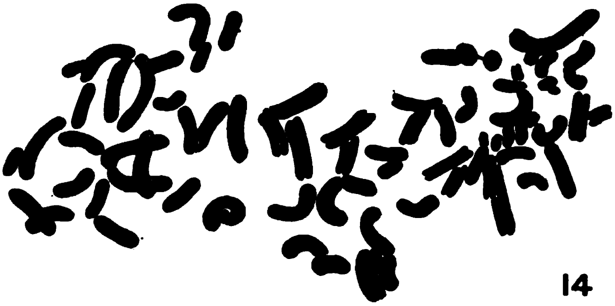
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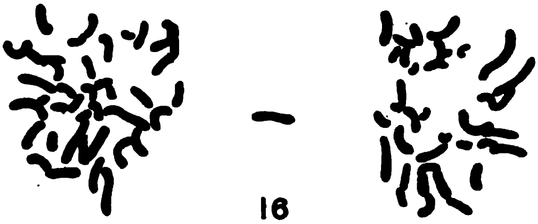
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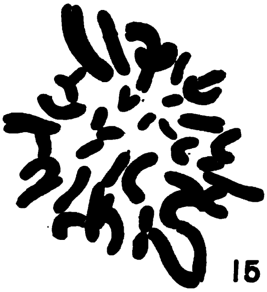
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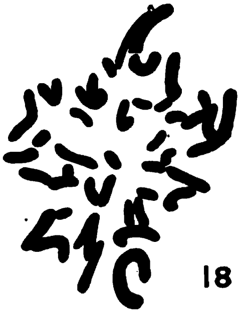
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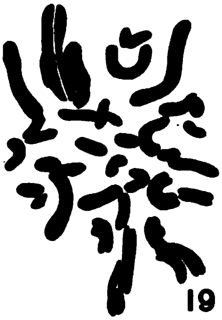
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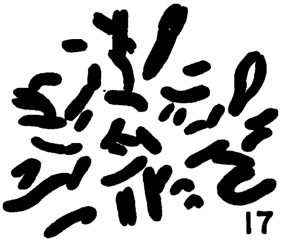
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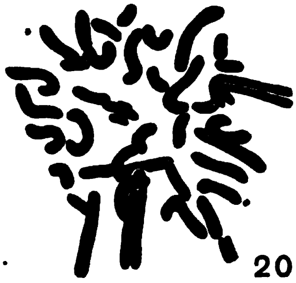
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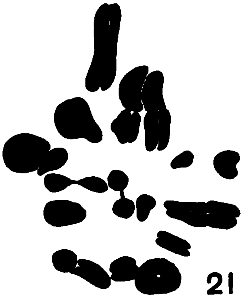
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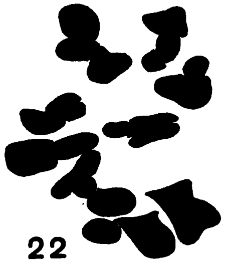
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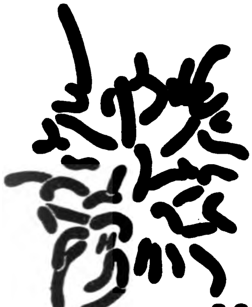
PLATE 3

EXPLANATION OF FIGURES

Polar views of metaphase stages from the brain of a 10 mm. pig embryo

FIGURE NUMBER	NUMBER OF CHROMOSOMES
23	40
24	42
25	43
26	46
27	48
28	47
29	49
30	50
31	50
32	52
33	54
34	54



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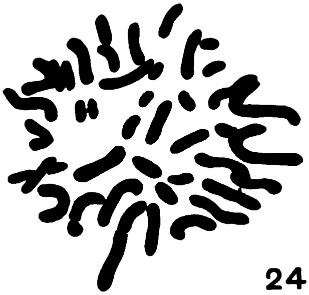

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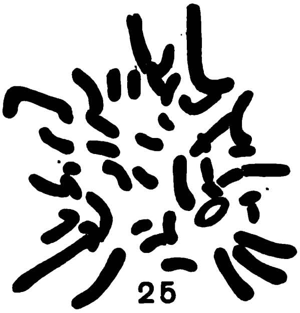

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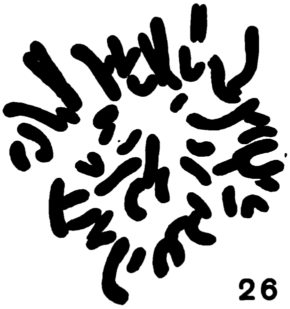

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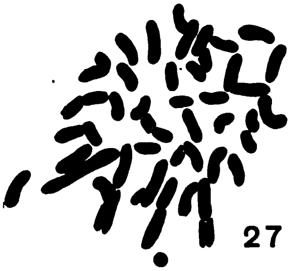

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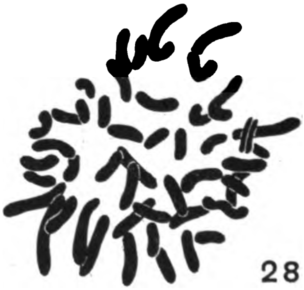

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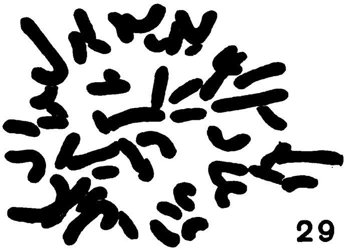

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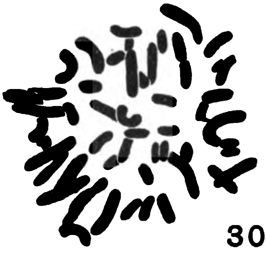

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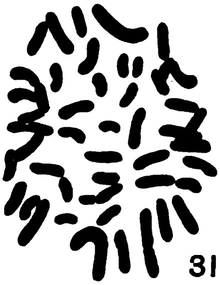

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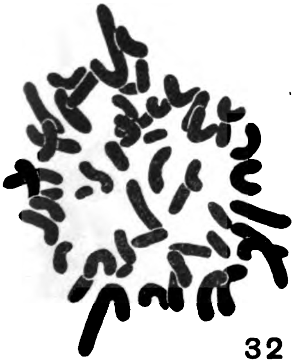

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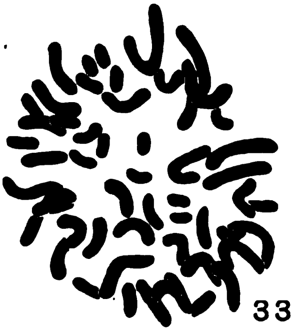

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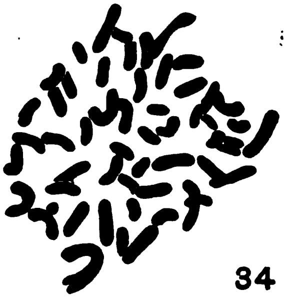


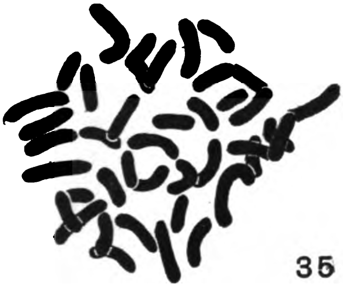
34

PLATE 4

EXPLANATION OF FIGURES

Polar views of metaphase stages from blood cells of pig embryos
35 to 45 From a 10 mm. pig embryo
46 From a 15 mm. pig embryo

FIGURE NUMBER	NUMBER OF CHROMOSOMES	REMARKS
35	41	Count not certain
36	41	Count not certain—note thin section in chromosome to left of number. May be possible point of fragmentation
37	42	
38	43	
39	44	
40	45	
41	47	
42	47	
43	49	
44	50	
45	55	
46	73	Very uncertain—the number of chromosomes is at least as many as indicated



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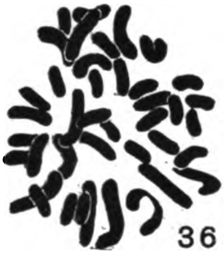

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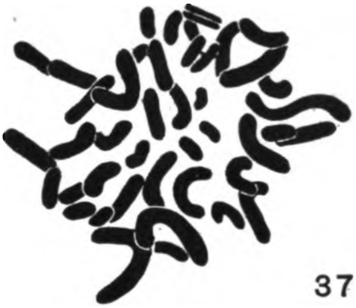

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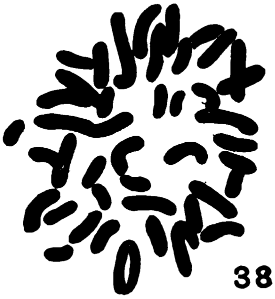

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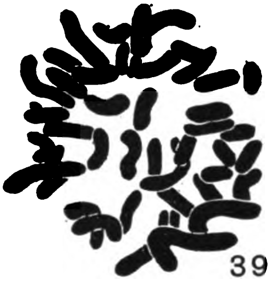

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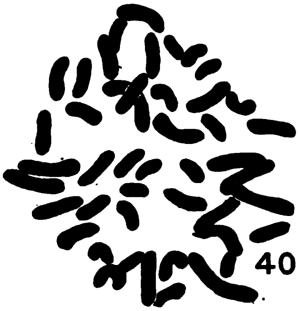

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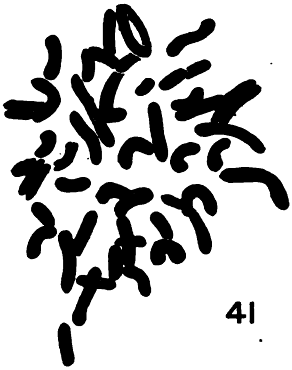

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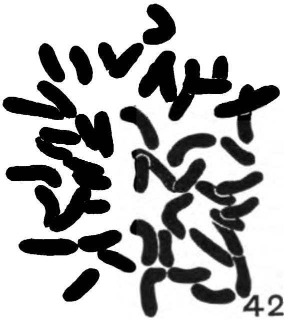

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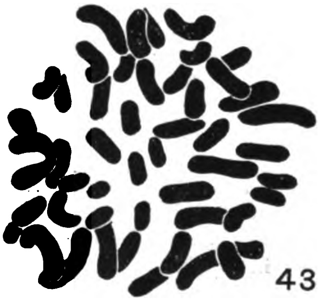

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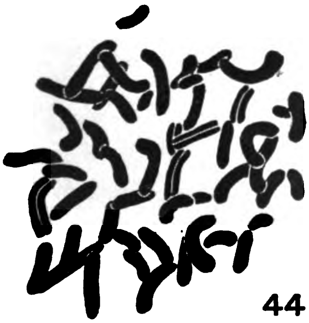

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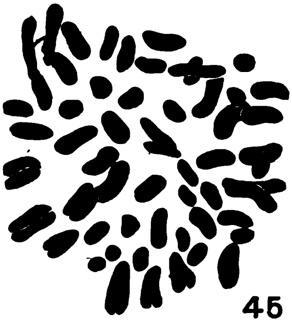

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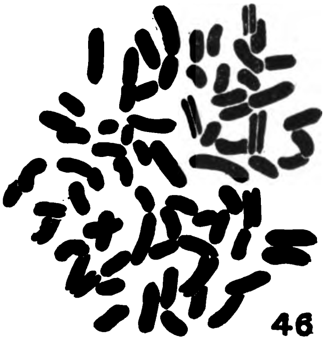

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PLATE 5

EXPLANATION OF FIGURES

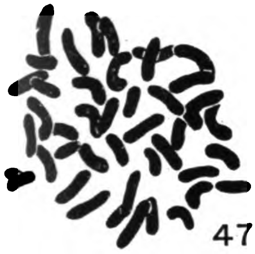
Polar view from metaphase stages from connective tissue cells
52 In late prophase

FIGURE NUMBER	NUMBER OF CHROMOSOMES	REMARKS
47	42	Count not certain—probably higher
48	43	
49	43	
50	45	Count not certain
51	47	
52	45	
53	49	
54	50	Count not certain
55	51	Count not certain
56	53	

57 The upper pole shows 43 chromosomes. Though the count is not certain there are at least as many chromosomes as figured. Since there are this number it is evident that the fragments must divide. The lower pole shows but 36 chromosomes but it was turned at an angle which made accurate drawing impossible.

THE DIPLOID CHROMOSOME COMPLEXES OF THE PIG PLATE 5

ROBERT T. HANCE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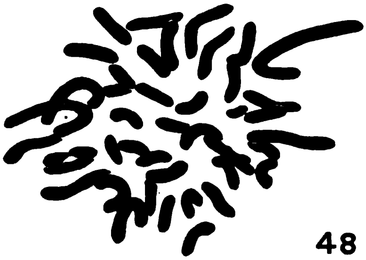
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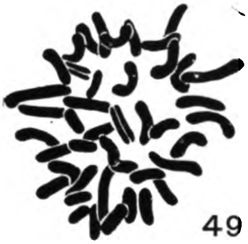
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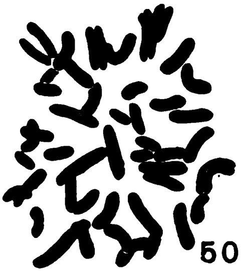
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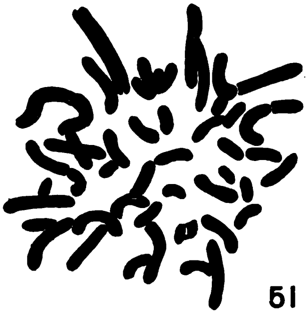
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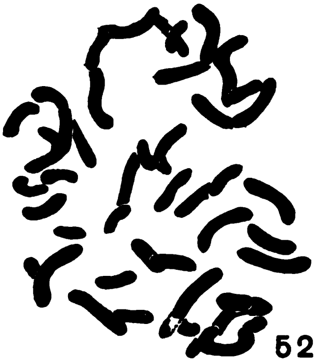
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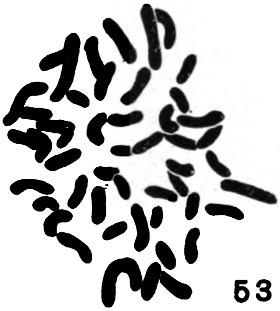
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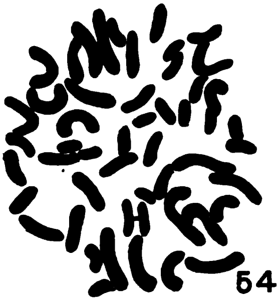
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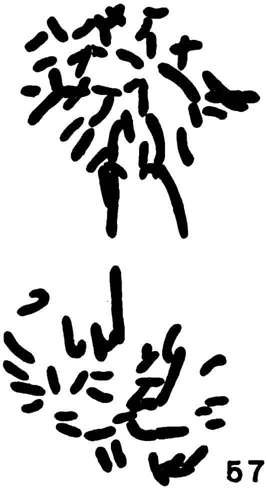
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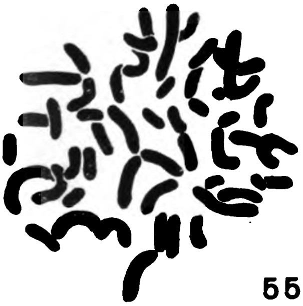
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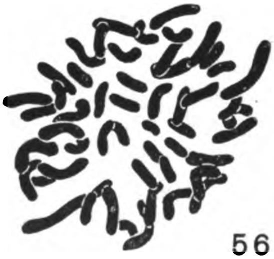
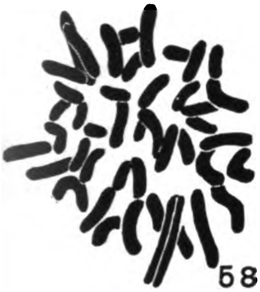

56

PLATE 6

EXPLANATION OF FIGURES

Polar views of metaphase stages from the cells of various tissues
From a 8 mm. pig embryo.

FIGURE NUMBER	TISSUE	NUMBER OF CHROMOSOMES	REMARKS
58	Diaphragm	44	Count uncertain
59	Limb bud	45	Count uncertain
60	Limb bud	48	
61	Limb bud	49	
62	Limb bud	50	
63	Limb bud	52	
64	Myotome	44	
65	Myotome	41	
66	Heart	43	
67	Myotome	43	
68	Mesoderm	54	Probably a giant cell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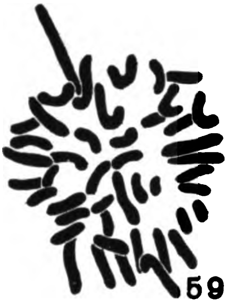
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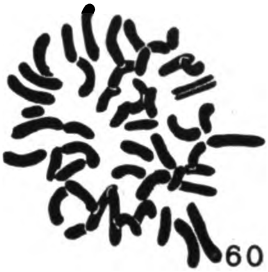
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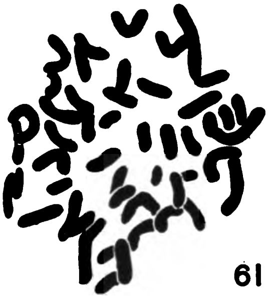
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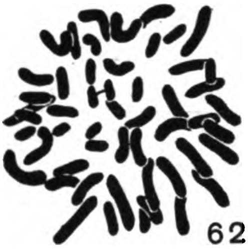
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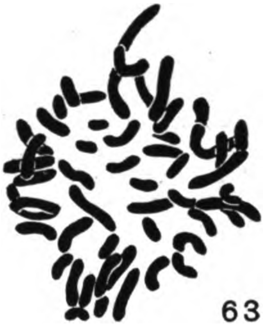
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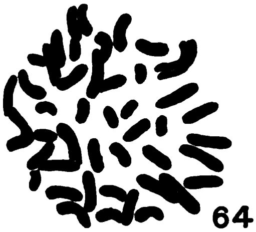
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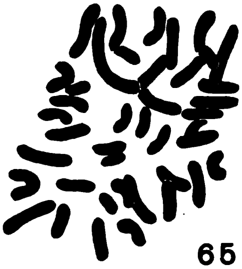
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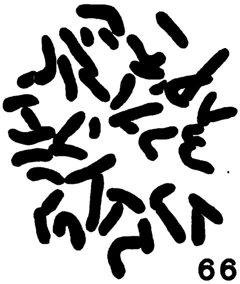
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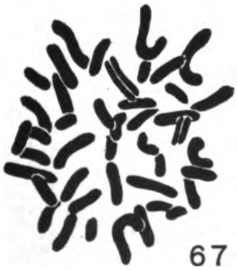
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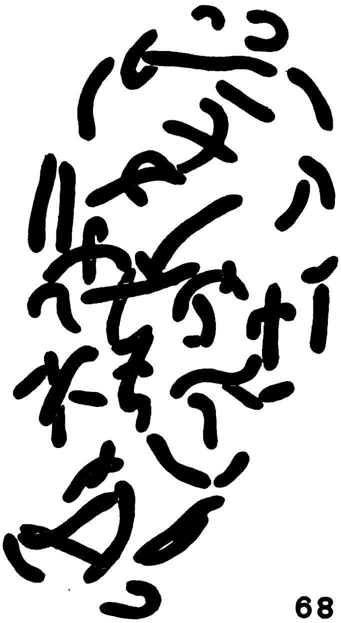

68

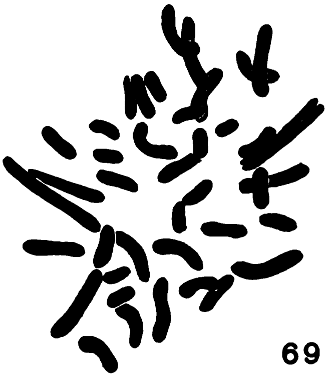
PLATE 7

EXPLANATION OF FIGURES

Polar views of metaphases in cells of various tissues

69 and 70 From 15 mm. pig embryo. Other figures from 8 mm. pig embryo.

FIGURE NUMBER	TISSUE	NUMBER OF CHROMOSOMES	REMARKS
69	Amnion	40	
70	Amnion	41	
71	Amnion	50	
72	Liver	47	
73	Liver	53	
74	Liver	45	
75	Wolffian body	41	
76	Wolffian body	45	
77	Wolffian body	48	
78	Lung	55	
79	Optic cup	53	Count uncertain
80	Umbilicus	46	



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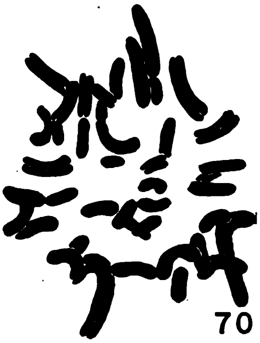

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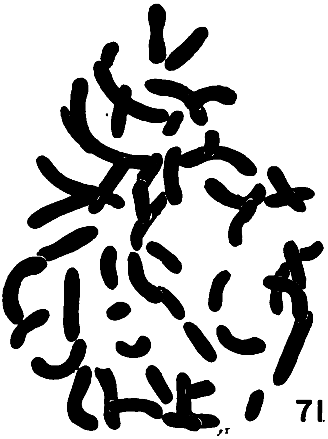

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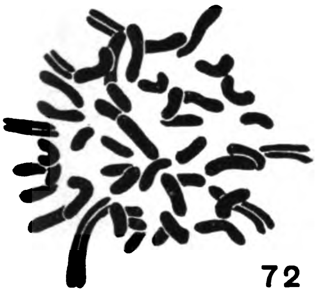

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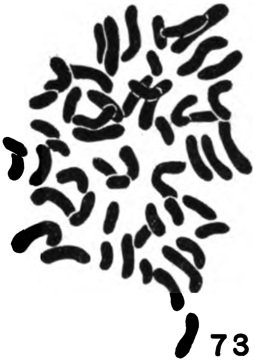

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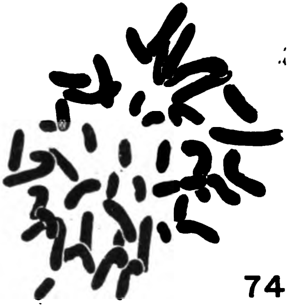

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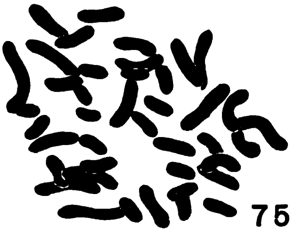

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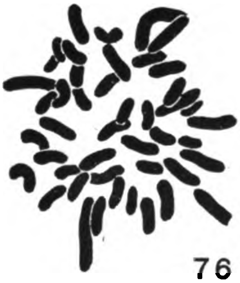

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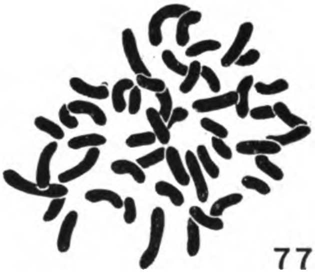

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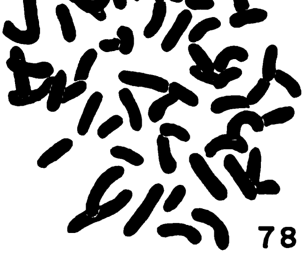

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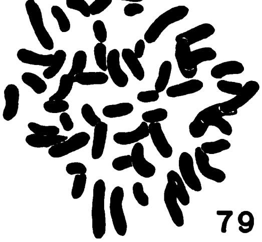

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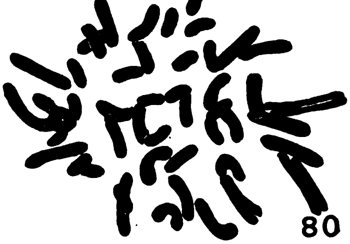


80

PLATE 8

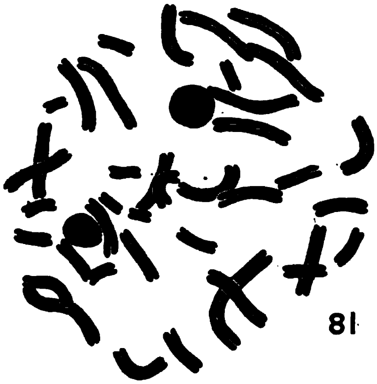
EXPLANATION OF FIGURES

81, 82, 84, 85 and 88 Drawn from amnion fixed in Flemming's weak solution.

83, 86 and 87 Drawn from amnion killed in Flemming's strong solution.

FIGURE NUMBER	NUMBER OF CHROMOSOMES
81	40
82	53
83	40
85	44

84, 86, 87 and 88 Show the structure of the chromosomes as they pass the poles and are discussed in the text on page 163.



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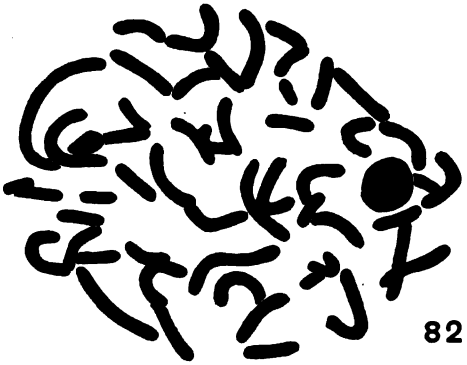

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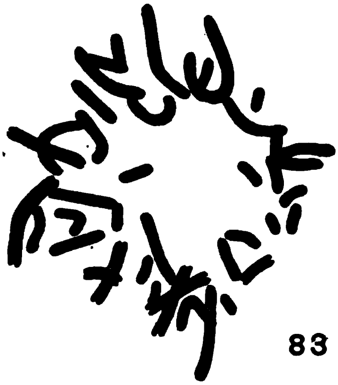

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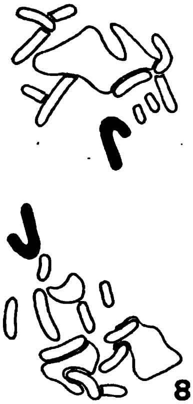

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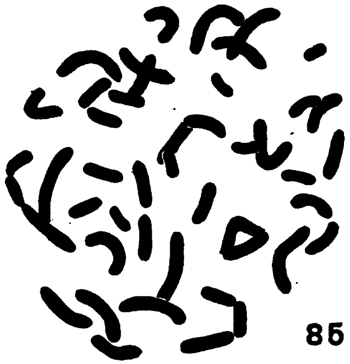

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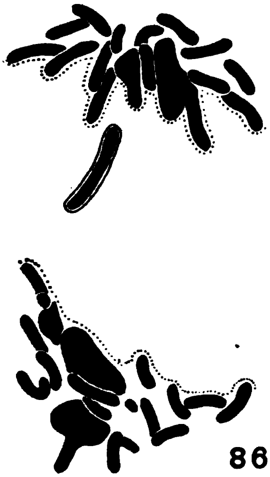

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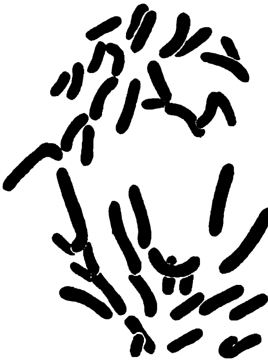

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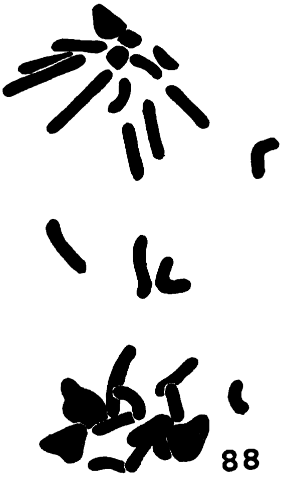


88

PLATE 9

EXPLANATION OF FIGURES

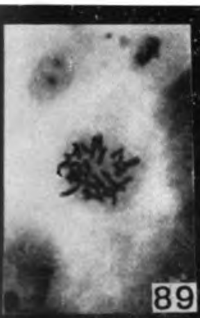
In preparing these photomicrographs a Zeiss 1.5 mm. apochromatic objective and a 4 X compensating ocular were used. The magnification at the level of the plate was about 1000 diameters. Seeds Process Plates were used. The photographs were made with the aid of artificial illumination. The photomicrographs appear in reproduction at the original magnification.

FIGURE NUMBER	TISSUE	NUMBER OF CHROMOSOMES	SAME CELL AS FIGURE NUMBER
89	Amnion		
90	Amnion		
91	Amnion	53	82
92	Amnion	45	
93	Amnion	50	
95	Amnion	50	
96 and 97	Spermatogonia	40	4 and 5
98	Brain	42	24
99	Blood	55	45

89, 92, 93 and 95 Fixed in Flemming's weak solution.

90, 91, and 96 to 99 Killed in Flemming's strong solution.

94 Illustrates the results obtained in poor fixation. This is a photograph of a group of poorly fixed spermatogonial cells in the testes of a pig.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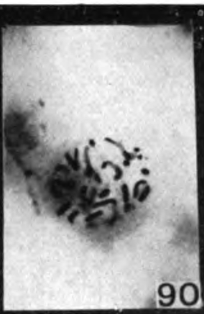
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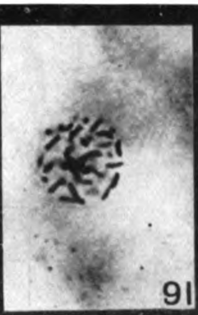
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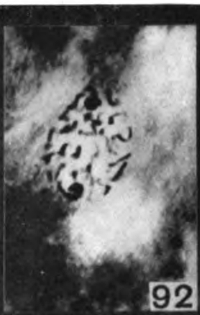
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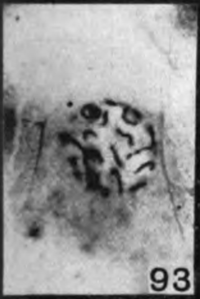
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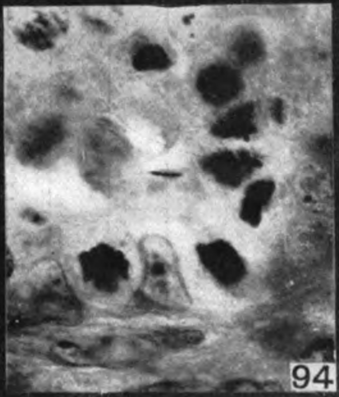
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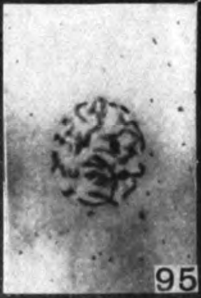
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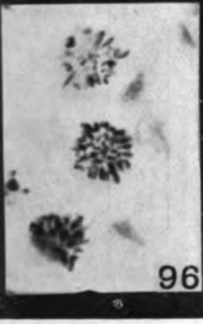
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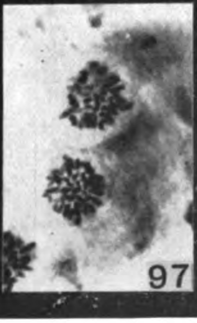
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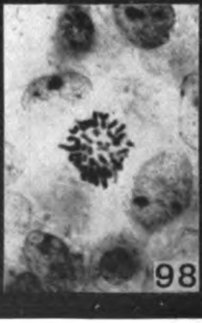
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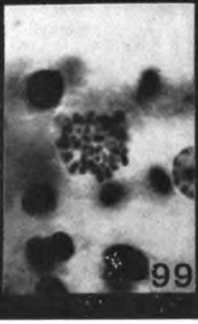

99

PLATE 10

EXPLANATION OF FIGURES

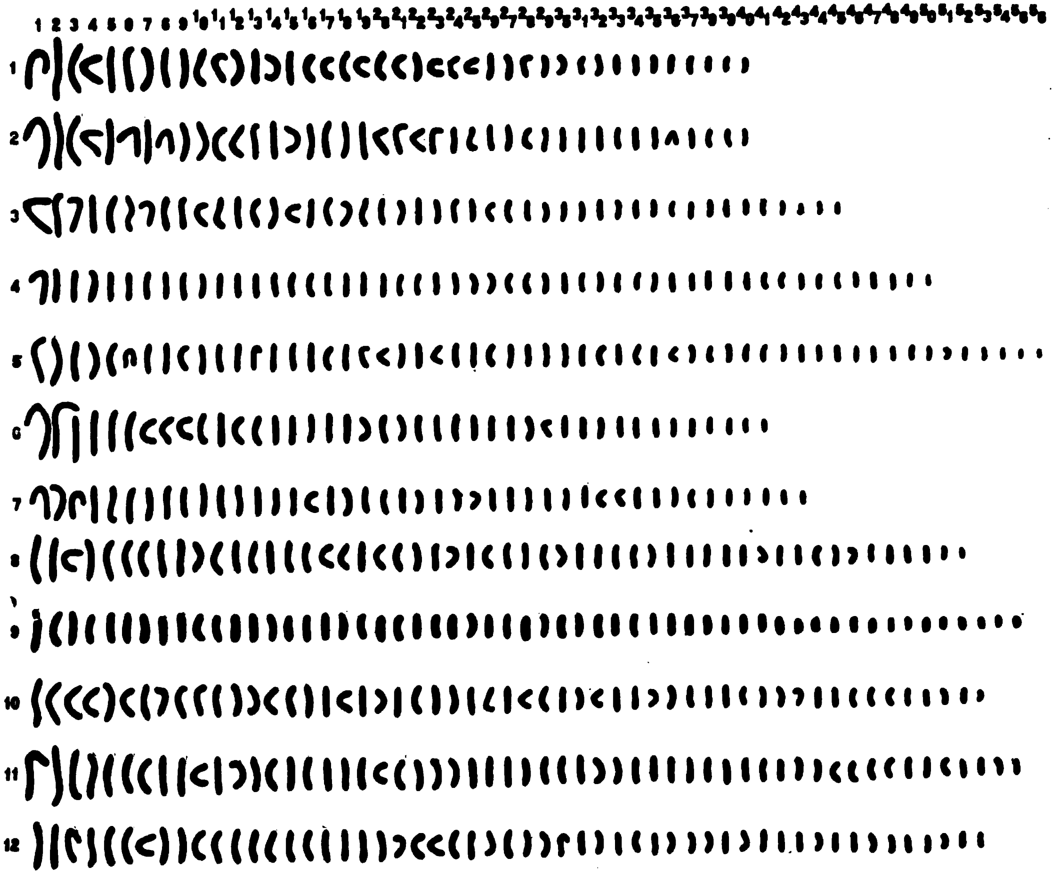
See table giving the length in millimeters of the chromosomes.

The chromosomes were enlarged to 20400 diameters before being measured. Under 'figure number' is given the number of the complex as it appears on previous plates. The number of the individual chromosomes as they appear on plate 10 is given in the columns to the left.

FIGURE NUMBER	5	23		31		70	67	63	45	56	78	73
Tissue........	Spermatogonium	Brain	Brain	Brain	Brain	Amnion	Myotone	Limb Bud	Blood	Connective	Lung	Liver
Row Number..	1	2	3	4	5	6	7	8	9	10	11	12
1	70	76	65	49	55	83	54	52	44	52	68	50
2	67	58	47	35	53	75	45	47	39	50	65	47
3	56	55	46	35	43	50	43	46	36	46	55	46
4	50	52	40	35	40	48	40	46	33	45	49	45
5	49	49	40	30	38	45	40	44	32	44	45	43
6	46	49	38	29	38	42	39	41	32	40	43	42
7	46	46	37	29	33	40	38	40	11	38	43	41
8	45	45	36	29	32	39	37	40	31	38	42	41
9	42	43	34	29	31	37	35	39	30	38	40	39
10	41	43	34	27	31	35	36	38	30	37	40	38
11	40	43	34	27	30	35	34	37	29	35	38	37
12	40	43	34	26	30	34	33	36	29	35	38	37
13	36	40	33	26	30	34	31	35	29	35	37	36
14	35	40	33	26	30	32	30	35	28	33	37	35
15	35	40	31	26	30	32	30	34	28	34	37	34
16	32	39	31	26	29	31	30	33	28	34	35	34
17	31	39	30	25	28	31	30	33	28	33	35	34
18	31	39	30	25	28	31	29	33	27	33	35	34
19	31	39	28	25	28	30	28	32	27	32	34	34
20	31	38	28	25	27	30	27	32	27	31	33	34
21	30	38	28	24	27	30	27	31	26	31	33	32
22	30	33	28	24	27	29	26	30	26	30	33	32
23	30	33	26	24	26	28	26	30	25	30	33	32
24	30	30	26	23	26	28	26	29	25	30	33	31
25	30	29	25	23	26	27	25	28	25	29	32	31
26	28	28	25	22	25	26	25	28	25	28	31	31
27	28	28	24	22	25	26	25	28	25	28	30	31
28	28	28	23	22	25	26	25	27	25	27	30	29
29	23	27	23	22	25	24	24	27	25	27	30	28
30	21	26	21	22	25	23	24	26	24	26	30	27
31	21	25	21	20	23	23	23	26	24	25	30	26
32	21	25	21	20	23	23	23	26	24	25	29	26
33	20	23	21	20	22	22	23	26	23	25	28	25

(Continued on page 222)

PLATE 10


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56

(Continued from page 220)

FIGURE NUMBER	5	23		31		70	67	63	45	56	78	73
Tissue	Spermatogonium	Brain	Brain	Brain	Brain	Amnion	Myotone	Limb Bud	Blood	Connective	Lung	Liver
Row Number	1	2	3	4	5	6	7	8	9	10	11	12
34	20	23	20	20	22	22	22	25	23	25	27	24
35	19	23	18	20	22	21	21	25	22	24	27	24
36	19	23	18	20	21	20	20	25	22	24	27	24
37	19	22	18	19	20	19	20	23	21	23	27	24
38	17	20	18	19	20	18	20	23	20	23	26	23
39	16	20	18	19	19	17	18	23	20	23	26	23
40	15	18	17	19	19	15	18	22	20	22	25	23
41			17	18	19	12	17	22	19	22	25	23
42			16	18	19		16	21	19	22	24	22
43			14	18	19		14	21	17	22	24	22
44			13	17	18			21	15	22	23	21
45			13	17	18			21	15	21	23	21
46				17	18			19	15	21	23	20
47				17	18			19	15	20	23	20
48				16	16			19	14	17	22	20
49				14	16			19	14	17	22	19
50				10	15			19	14	17	21	18
51					15			15	13	17	21	18
52					14			12	13	16	20	17
53					14				12	12	18	16
54					12				12		18	
55					10				11		17	
56					9							

University of Pennsylvania

STUDIES ON THE OLFACTORY BULBS OF THE ALBINO RAT

PART I. EFFECT OF A DEFECTIVE DIET AND OF EXERICISE
PART II. NUMBER OF CELLS IN BULB

BY
CAROLINE MAUDE HOLT

A THESIS

Presented to the faculty of the graduate school
in partial fulfillment of the requirements
for the degree of Doctor
of Philosophy

1917

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February, 1917

STUDIES ON THE OLFACTORY BULBS OF THE ALBINO RAT—IN TWO PARTS

I. EFFECT OF A DEFECTIVE DIET AND OF EXERCISE

II. NUMBER OF CELLS IN BULB

CAROLINE M. HOLT

From The Wistar Institute of Anatomy and Biology[1]

FOUR PLATES

PART I. EXPERIMENTS TO DETERMINE THE EFFECT OF A DEFECTIVE DIET AND OF EXERCISE UPON THE WEIGHT OF THE OLFACTORY BULBS

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I. INTRODUCTION

The various members of the mammalian series show considerable variation in the relative development of all parts of the central nervous system, but probably no part of the encephalon shows so great a degree of variability as does the rhinencephalon. Of this portion of the brain, the olfactory bulbs are, without doubt, the most variable in size. Thus we have the very large bulbs of the opossum and the ant-eater; the almost rudimentary bulbs of the ape and of man; extreme reduction of these organs in the Cetacea, with their complete disappearance in the dolphin. Not only do we find variation in size of the olfactory bulbs among the different orders of mammals, but we find that there is a considerable degree of variability within each order and even among the members of the same species.

This variation in size and weight of the olfactory bulbs within a species is well illustrated by observations upon the rats in the colony of The Wistar Institute. The domesticated albino rats exhibit a considerable range in the development of this part of the brain. But while we find an appreciable difference in the bulb size of rats of different litters even under like environmental conditions, the individuals of a given litter usually show a more uniform development of the olfactory system. Some wild Norway rats examined at The Wistar Institute a few years ago had olfactory bulbs heavier in proportion to total brain weight than the bulbs of the albino. In the course of the present study, observations made upon some thirty wild Norway rats caught at different places in Philadelphia suggested that this difference between the two strains is not a constant one, for, while the olfactory bulbs of these animals were much heavier than those of the albinos, the ratio between bulb weight and brain weight in this series was about the same in the two forms.

Inequality in the size of the two bulbs in the same individual appears not infrequently in the albino and when it occurs it will often be found in several, and occasionally in all, members of the same litter. For this reason, in selecting material for these experiments, we discarded all litters in which cases of asymmetry were observed among the initial controls.

Observations made from time to time by Dr. Donaldson, indicated that rats born in the early summer differ from winter-born rats in the relative size of the olfactory bulbs; also that there might be a difference between rats reared on a restricted diet, such as is frequently used in colonies, and those fed on the table-scrap diet adopted for the Wistar colony. Moreover, cases had appeared in which the bulbs of sick rats were apparently smaller than those of healthy individuals.

All these facts suggested that there might be factors in the living conditions of the rats which would account for the variability of this portion of the nervous system, the growth of the bulbs being retarded or arrested in rats reared under unfavorable conditions, such as the intense heat of the summer, or a monotonous diet, or in those suffering from the various infections which may attack the rats from time to time.

It was, therefore, with the hope of throwing some light upon the question of the effect of environmental conditions upon the olfactory bulb of the growing albino rat, that, at the suggestion of Dr. Donaldson, the present experiments were undertaken. The problem resolved itself into two questions—Can the growth of the olfactory bulbs of the stock albino be modified (1) by underfeeding or (2) by exercise?

The writer wishes here to express her deep gratitude to Dr. Donaldson for his unfailing helpfulness and encouragement, and her appreciation to Dr. Stotsenburg and Dr. Heuser, and to the other members of The Wistar Institute who did much to aid in the course of the experiments which have extended over the past two years.

II. DEFECTIVE DIET EXPERIMENTS

1. *Previous experiments on the effect of starvation upon the central nervous system of the rat*

There have been several previous studies upon the effect of underfeeding and of starvation upon the central nervous system of the albino rat. In 1904, Hatai reported experiments on 'partial starvation' for twenty-one days. He fed large quantities of starch with some fat but no proteids of any kind. He obtained a deficiency in body weight of 27 per cent in females and 32 per cent in males. Taking the values from the initial controls for a standard, the brains of the test rats showed, at the end of twenty-one days, a deficiency of 2.8 per cent for the females and 5.8 per cent for the males. Thus the treatment produced not only an arrest of brain growth but a loss in absolute brain weight. This experiment was followed by a series in which the animals, after a defective diet (Oswego starch), were returned to a normal diet. Here Hatai ('07) found that the effect of twenty-one days of partial starvation was eventually compensated for, so far as brain weight was concerned, but the central nervous system had suffered some change in its chemical composition. The following year ('08), Hatai published the results of further experiments, this time in quantitative underfeeding with an adequate ration, in which he concludes that growth in the stunted rats is just as normal as in the controls; i.e., all parts are proportionately stunted.

In 1911, Donaldson published an account of the effect of underfeeding, with a quantitatively deficient, but adequate ration, on the percentage of water, on the ether-alcohol extractives and on medullation in the central nervous system of albino rats, showing a slight diminution of percentage of water, slight increase in percentage of ether-alcohol extractives, and no notable difference in medullation.

Jackson ('15) found, in young rats maintained at a constant weight on a diet of bread and milk, that the relation between body weight and brain weight remained unchanged. The brain ceased to grow simultaneously with the body, while the cord increased somewhat in weight during underfeeding.

Although there are, at present, no data by which it is possible to make a definite comparison between the effect of a defective diet and that of an adequate, but quantitatively insufficient diet, it is important to bear in mind in each case the method by which growth has been retarded or arrested.

Two series of experiments upon the effect of underfeeding were undertaken—one upon rats which had been reared to the time of weaning (at three or at four weeks of age) by well-fed mothers, Series A; the other, upon rats reared to the time of weaning by underfed mothers, which meant rats underfed practically from birth, Series B.

Observations were also made upon a few sick animals, Series C.

2. Series A. A_1, rats underfed from time of weaning at eighteen to twenty days, and A_2, at thirty to thirty-two days

a. Method. As has been previously stated, while there is a considerable range of variation between litters in the matter of the relative size of the olfactory bulbs, yet within a given litter the size is fairly uniform. For this reason, so far as possible, control and test animals were taken from the same litter. This, of course, made it necessary to select fairly large litters in order to have several animals for initial and final controls, and also for experiment. The litters were always taken from healthy stock animals.

For the first few individuals experimented upon, no initial controls were examined, but the results of these experiments made the advisability of such controls apparent and subsequently each litter was weighed and divided into three groups; so far as possible, equivalent in sex, weight, and bodily condition. All these rats were ear marked and a card filed for the data upon each animal.

The first or initial control animals were at once etherized, weighed, measured, eviscerated, and the brains removed. One olfactory bulb was cut off from each brain, in the following manner. The brain was placed, ventral side down, on the dissecting board. Then with a thin, sharp scalpel held in a position perpendicular to the plane of the board and at right angles to the plane of the median longitudinal fissure, the bulb was severed just below the anterior limit of the cerebrum.[2] The bulb, with the remainder of the brain was then placed in a covered weighing bottle and the weight of both the entire brain and of the severed bulb ascertained.

The final controls were weighed and placed under the normal living conditions of the colony: i.e., housed, in long wooden cages with wire fronts, thick shaving-covered floors, and paper nests, and given plenty of fresh water with a carefully supervised scrap diet. The test rats were weighed and placed in adjoining cages under exactly the same conditions as the final controls, save for the diet. The food given the test rats consisted of an unlimited amount of whole corn, usually fed on the cob, save in case of very young animals, or those weak from a long period of underfeeding. In such cases, the corn was shelled as the animals were not able to remove a sufficient amount for themselves.

Both control and test animals were weighed from time to time and the weights recorded. Note was also made of any irregularities, such as a temporary change in diet, etc.

At maturity, a certain number of test and of control animals were mated in order to find out whether underfeeding affected the fertility of albino rats.

In the case of relatively small litters in which the members were usually well grown and in good physical condition when weaned—and especially if weaning was delayed until the rats were four weeks old—it was possible to keep the test animal on a corn diet for a month or more with practically no difficulty.

[2] Small bulbs tend to differ characteristically in shape from large ones. On section it is seen that the cap of gray substance extends somewhat further caudad on the ventral surface of the small bulb than it does in the case of the large bulb. The weight of gray substance thus lost in the case of the small bulb is a very small fraction of the total weight of the bulb but a much larger fraction of the gray cap. Care must, therefore, be taken to include this portion when the number of cells of the gray substance is to be determined.

With the rats weaned at three weeks or in case of small rats from very large litters, there was a good deal of trouble in keeping the animals on the corn diet for so long a time, and of course the difficulty increased as the period of underfeeding was prolonged.

At first an attempt was made to keep animals from several litters in one cage with the result that after a short time, the less well grown rats were killed and eaten by the stronger individuals. Then the plan was adopted of having members of only one litter in a cage. This worked successfully up to the time when the animals began to weaken. Then the males frequently killed and ate the females. So finally, for prolonged experiments, it was found safer to place only animals of the same sex, approximate weight and physical condition together, but even this precaution was not always sufficient.

In most cases, for the first few weeks, there was a very slow gain in weight or the weight was just maintained. But in every case when an animal began to lose or became very feeble, a dose of condensed milk was fed. One or two doses were usually sufficient to restore the animal to equilibrium and there was not infrequently a sudden temporary gain in weight, doubtless due to increased appetite and the consequent gorging of the alimentary tract with corn.

In a few cases where the underfeeding had gone on for several months, it became necessary to administer small doses of condensed milk more frequently—in two cases, practically every day—in order to keep the animals from losing weight.

At the end of the experiment, both test and final control animals were killed, weighed, measured, eviscerated, and brains and bulbs weighed as in the case of the initial controls. One bulb with a part of the cerebrum was preserved for histological study. A record was kept of any signs of disease or other abnormality. The weighing was done in closed bottles and all weights of brain and of olfactory bulbs were made to 0.1 mgm., but recorded here in milligrams only.

b. Results. General morphological and physiological modifications. A summary of the data from observations upon 108 individuals of Series A is given in tables 1 to 8. The complete tables with the records for each individual rat of this, as well as of the other series, are deposited at The Wistar Institute. Of the two litters weaned at eighteen and twenty days, only three individuals survived to be killed; the others died in the cages and the brains were not weighed. The records for the three rats just named have been included in tables 3 and 7, and their controls, with the corresponding controls. The size and body weight of rats weaned at the end of the third week and placed on a corn diet indicated clearly that under like conditions, rats weaned at three weeks are considerably more sensitive to adverse conditions than are those weaned at four weeks.

For every individual of Series A_1 and A_2 (tables 1 to 8), the stunting effect of the corn diet was apparent almost from the first. During the early weeks of underfeeding the test rats appeared rather more lively than the controls. Later this activity decreased, the gait became unsteady, and the animals appeared stupid. They were often unable to find the dish of condensed milk by themselves, whereas control rats would go to it immediately. This suggests that the underfed animals lacked an acute sense of smell and perhaps did not see clearly.

In every one of the test animals of which there are complete records, the general bodily growth was arrested by a diet of corn. This agrees with the observations of Osborne and Mendel ('13). These rats remained like young animals in appearance as well as in size. The earlier weaning took place and the corn diet was begun, the more complete the stunting.

The skeleton became modified and somewhat distorted owing to imperfect calcification. The growth of the long bones was not quite so completely arrested as that of the rest of the skeleton. The skull, sternum, and sometimes the ribs, became like parchment. In two cases the pressure of the heart upon the sternum had formed a sort of pocket out of that structure, which appeared like a tumor on the ventral side of the rat. The vertebral column became somewhat bowed, giving to the rat a 'humped' appearance and making it necessary to stretch the animals when measuring body length. One to four months of underfeeding, following the first month under normal conditions, left the rats but slightly longer (4 to 10 mm.) than the initial controls measured at thirty days. The average increase in weight was in about the same proportion. Compare tables 2 to 8, for body weight and body length.

All the rats showed extreme emaciation but this condition was largely masked by the condition of the coats. The hair remained short and soft, with a fluffiness which gave even to mature rats the appearance of plump young animals. Such emaciation was, of course, accompanied by great muscular weakness. Rats kept for long periods on the defective diet became unable to remove corn from the cobs. They walked with a tottering gait and moved about but little.

The cyanosed condition of these animals was clearly indicated by the blue color of all exposed parts of the body—nose, ears, feet and tail. In protracted cases of underfeeding, a chronic palpitation of the heart developed which increased in violence as time went on. As a result of this, the whole body shook constantly.

All animals kept on corn up to maturity failed to breed or to show any sexual instinct whatever.

Effect on brain and olfactory bulbs (compare tables 1 to 8). In Series A, both A_1 and A_2 show a slight increase in brain weight during the period of underfeeding. Under normal conditions, as the rat grows, the brain becomes relatively lighter in proportion to body weight. In the underfed rats the brain forms practically the same proportion of the total body weight as in the initial control rats (agreeing with Jackson's results ('15)), which of course indicates in the cases where growth has taken place that the brain has not been as much arrested in its development as has the rest of the body.

After four to eight weeks of underfeeding, the rats of Series A_1 and A_2 had olfactory bulbs which, taken together, formed about the same proportion of the total brain weights as did the bulbs of the initial controls of the same series, showing that the relation of these parts of the brains had not been changed during the experiment. But normally the olfactory bulbs grow faster during this period than the rest of the brain so that at eight weeks, for example, the bulbs should form a considerably greater percentage of the total brain weight than at thirty days. The average absolute weight of the bulbs of the test animals was equal to but 70 to 81 per cent of the average weight of the bulbs in the control animals of the same series. It is therefore evident that the retarding effect of underfeeding has been greater upon the olfactory bulbs than upon the other parts of the brain, which had 85 to 90 per cent of the weight of the brains in the control series.

If the relative weight of the bulbs in Series A_1 and A_2 is determined for the test group as contrasted with the final control group, we obtain the following relations:

TABLE 1

	GROUP	AGE	PERCENTAGE WEIGHT OF OLFACTORY BULBS
		days	
Table 3..........	Test rats, defective diet	60	3.52
Table 4..........	Final controls	60	3.99
Table 5..........	Test rats, defective diet	79	3.39
Table 6..........	Final controls	79	4.16
Table 7..........	Test rats, defective diet	118	3.83
Table 8..........	Final controls	128	4.30

This arrangement of the results shows clearly that in each of the three sets, grouped according to age, the olfactory bulbs of the underfed rats are significantly lighter in proportional weight than those of the controls. We may, therefore, conclude that the relative weight of the olfactory bulbs is reduced by the form of defective feeding employed in this experiment.

The details are given in tables 2 to 8, which follow.

3. *Series B. Rats on deficient diet from birth*

a. Method. Since it was evident that the earlier the animals were weaned, the greater the stunting effect of a qualitatively inadequate diet, it occurred to the writer that it would be interesting to try underfeeding from birth, by underfeeding the

TABLE 2. SERIES A

Initial control animals

In all of the tables the averages are weighted for the number of animals in each entry

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
14 males	30	44.1	118	1.439	0.051	3.52	3.01–3.97
11 females	30	43.5	116	1.409	0.050	3.53	2.41–4.19
Averages for males and females		43.8	117	1.426	0.050	3.53	2.41–4.19

TABLE 3. SERIES A

Test animals

Stock albinos kept on corn diet for twenty-nine to forty-two days after weaning at three to four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
15 males	60	55.5	126	1.505	0.052	3.45	2.38–4.53
11 females	60	53.9	126	1.502	0.054	3.62	2.68–4.21
Averages for males and females		54.8	126	1.504	0.053	3.52	2.38–4.53

TABLE 4. SERIES A

Final control animals

Stock albinos kept on normal diet for twenty-nine to forty-two days after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
19 males	61	127.4	167	1.668	0.066	3.95	2.76–4.62
7 females	60	95.1	154	1.606	0.065	4.04	3.66–4.53
Averages for males and females		118.8	164	1.651	0.066	3.99	2.76–4.62
Summary $\frac{\text{Test}}{\text{Control}}$			77	91	81		

TABLE 5. SERIES A

Test animals

Stock albinos kept on corn diet for forty-nine days after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
2 males....................	80	47.8	126	1.502	0.055	3.63	3.31–3.93
1 female...................	78	37.8	116	1.458	0.042	2.89	
Averages for males and females..................			124	1.487	0.050	3.39	2.89–3.63

TABLE 6. SERIES A

Final control animals

Stock albinos kept on normal diet for forty-nine days after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
2 males....................	80	155.0	178	1.727	0.068	3.93	3.72–4.14
1 female...................	78	151.2	183	1.703	0.079	4.63	
Averages for males and females..................		153.8	180	1.719	0.072	4.16	3.72–4.63
Summary $\frac{\text{Test}}{\text{Control}}$.........			69%	78%	70%		

TABLE 7. SERIES A

Test animals

Stock albinos kept on corn diet for fifty-nine days or more, after weaning at four weeks. (One rat weaned at eighteen days)

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
7 males....................	120	47.1	118	1.463	0.057	3.86	3.47–4.19
6 females..................	115	54.3	126	1.594	0.060	3.79	3.55–4.29
Averages for males and females..................		50.5	122	1.524	0.058	3.83	3.47–4.29

TABLE 8. SERIES A

Final control animals

Stock albinos kept on normal diet for fifty-nine days or more, after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
8 males	121	201.4	199	1.806	0.078	4.29	3.81–4.73
4 females	136[1]	157.0	181	1.706	0.074	4.32	4.03–4.62
Averages for males and females		186.7	193	1.772	0.076	4.30	3.81–4.73
Summary $\frac{\text{Test}}{\text{Control}}$			63%	85%	77%		

[1] This higher average age for the female controls is due to the fact that one female was kept for breeding purposes until two hundred and thirteen days old. As all her measurements were practically identical with those of another female of same litter, one hundred and fifteen days old, the record was included in the table.

mothers which were bearing or nursing the young to be tested. Consequently nine pregnant females were selected. A few were put on a corn diet several days before the birth of the young, but most of them began the corn feeding on the day of the birth of the litter. The young rats were weaned at three weeks and fed exclusively on corn.

It is intended to carry out this experiment more extensively at some future time but enough animals were tested to give significant results. It was found very difficult to raise such litters, for two reasons. In the first place, after the young reached an age to leave the nest, the mother was very apt to kill the entire litter. This, apparently, was not because of hunger, for in all but two cases in which the young rats were partially eaten, the animals were mutilated only to the extent of a bite through the cerebellum, and sometimes through the front of the throat. It has been suggested that the increasing demands of the young, coupled with an inadequate milk supply, may have been the cause of this unnatural behavior of the mothers.

But the chief reason for the difficulty in raising these rats was their lack of vitality. Although very active and playful, these animals were extremely frail little creatures, so weak that the slightest disturbance was likely to prove fatal. For example, an unusually active and promising test rat of fifty-three days, was carried from the colony to the laboratory for examination. As he appeared much excited, the carrier cage was set aside for an hour. The rat was heard running about for a time but at the end of the hour was found dead. The body weight of this rat was that of an animal fifteen days old and the brain weight was scarcely more. Young rats might appear lively and in every way normal in a late afternoon and be found dead in the cage next morning, for no reason to be discovered even after careful examination. Of nine such litters only two survived to the time of weaning, and these were kept with much difficulty.

A litter of 'runts' was also included in this series. This was a litter of rats, all of which failed to grow normally, presumably because the mother had an insufficient supply of milk. They appeared in every way like the rats which had been stunted by underfeeding the mothers.

b. Results. The general results of underfeeding in Series B were essentially the same in character as in Series A but they were considerably more marked (tables 9, 10 and 11). The body length and general appearance of seventy-seven day rats, underfed from birth, were practically the same as in normal three-weeks-old rats, save for the extreme cyanosed condition.

From a comparison of Series A_1, A_2, and B, it becomes evident that it is easier to retard the growth of an eighteen day rat than of a rat thirty days old, and still easier to stop the growth of a rat at about the size of an eighteen day individual if the underfeeding is begun at birth. Moreover, it is obviously far more difficult to rear these animals underfed from birth than rats which have been allowed to get a good start of thirty days under favorable conditions and are therefore much more resistant to the deleterious effects of partial starvation.

Effect on brain and olfactory bulbs. Series B shows brains actually lighter in weight at twenty-four to fifty-three days of age than normal brains of seventeen days (The Rat, table 74). Rats seventy-seven days old had brains weighing practically the same as those of normal female rats of forty-two days.

Bulbs of rats twenty-four to fifty-three days old, actually weighed only 70 per cent as much as those of normal rats of thirty days (table 2) and only 52 per cent as much as bulbs of normal rats eight weeks old (table 4).

Rats eleven weeks old (table 11) gave bulbs of the same absolute weight as those of control rats of thirty days (table 2). In both cases the olfactory bulbs formed a smaller per cent of the total brain weight than appeared among the controls of like age in Series A, as the following arrangement of the data shows:

TABLE 9

	GROUP	AGE	PERCENTAGE WEIGHT OF OLFACTORY BULBS
		days	
Table 10.........	Test rats, defective diet-Series B.	24–53	3.14
Table 2..........	Control.	30	3.53
Table 11.........	Test rats, defective diet-Series B	77	3.48
Table 4..........	Control	60	3.99
Table 6..........	Control	79	4.16

The details for these series are given in tables 10 and 11 which follow.

4. Series C. Sick rats

In the course of the experiments a number of sick rats came under observation. Eleven of these were examined to determine whether the brain, and especially the olfactory bulbs, showed any effects of the diseased condition. Three of these rats were the sole survivors from a group of twelve attacked by a serious bowel trouble which killed the other nine occupants of the cages. At the time of the onset of the illness, the rats were about eighty days old. After about ten days, these three seemed to recover and were kept until they were about a hundred and thirty-five

TABLE 10. SERIES B

Test animals

Stock albinos underfed from birth. Under two months old

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
6 males	24–38	20.1	86	1.114	0.036	3.19	3.06–3.38
4 females	24–53	15.6	79	1.062	0.033	3.08	2.11–3.82
Averages for males and females		18.1	83	1.091	0.034	3.14	

TABLE 11. SERIES B 1.

Test animals

Stock albinos underfed from birth. Over two months old

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
3 females	77	31.2	102	1.437	0.050	3.48	3.15–3.93

days old when they were killed and examined. The other eight sick rats of this Series C were individuals showing a considerable infection of the lungs, and one of these (No. 20) had, in addition, a large abscess of the liver.

All of these rats were examined in the same way as those of Series A.

a. Results. In the group of sick animals, those with the intestinal infection had, at one hundred and thirty-four days, bulbs which averaged 0.050 gram or 3.02 per cent of the total brain weight (table 12, group 1) while a set of normal individuals of practically the same age gave an average of 0.073 gram or 4.32 per cent of the total brain weight (see table 20, group of females). These results seem especially interesting because here the adverse conditions appeared only after the rats were well grown—eighty days old—and lasted only about te[illegible]

The remaining two groups of sick rats all had inf[illegible]gs and were very old when killed. The two males [illegible] bulbs averaging 0.037 gram, or 2.08 per cent of the total brain weight (table 12, group 2); while for the four females, the bulbs averaged 0.033 gram, 1.89 per cent of the total brain weight (table 12, group 3). For these last two groups there are no data of normal individuals for comparison but the percentage for the bulbs is strikingly low. Some unpublished data in Dr. Donaldson's hands show, however, that while the relative weight of the olfactory bulbs tends to increase up to about one hundred and fifty days of age, in older rats there is a tendency to decrease so that some of this decrease observed in the old sick rats (groups 2 and 3) may be due to normal age changes. But the remarkably small proportional weight of the bulbs here examined is probably due chiefly to the effect of disease.

In this connection may be mentioned two young rats of litter PR (group 2), killed at seventy days. Each had infected lungs. These rats came from parents with infected lungs and had lived since birth in a dark damp cage. One had very small unequal bulbs which were not weighed. The other had bulbs weighing only 0.019 gram or 1.30 per cent of the entire brain weight. This pair of bulbs were the smallest observed in the whole series of experiments. It seems quite evident that the bulbs are abnormal and quite probable that this abnormality is due to disease.

5. Summary and conclusions. Defective diet experiments

1. General bodily growth in the albino rat is arrested by an exclusive ration of corn which constitutes a defective diet (Osborne and Mendel).

a. The skeleton is poorly calcified and somewhat distorted.
b. The muscular system is greatly reduced.
c. The coat has the appearance of that of a young animal.

2. Functional disturbances follow the arrested development.

a. There is increasing muscular weakness.
[illegible] ncreasing palpitation of the heart.
[illegible] imals appear cyanosed.

TABLE 12. SERIES C

Sick animals

Females

Group 1. Three albino rats from a lot of twelve controls for revolving cage experiment. At about eighty days all contracted a severe bowel trouble from which these three recovered.

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT
	days	*gm.*	*mm.*	*gm.*	*gm.*	
Z_1	135	128.5	165	1.607	0.033	2.03
Y_{22}	135	150.5	180	1.534	0.034	2.24
X_3	134	157.9	182	1.803	0.082	4.55
Average females	....	145.6	176	1.648	0.050	3.02

Males

Group 2. Two, old, with infected lungs.

PR_1	70	88	148	1.489	0.019	1.30
PR_4	70	90	155	1.538	unequal	
No. 24	old	213	202	1.759	0.050	2.81
No. 69	365	214	195	1.791	0.024	1.35
Average Numbers 24 and 69	....	213.5	198	1.775	0.037	2.08

Females

Group 3. Infected lungs, old age, and in case of No. 20, bad abscesses on liver.

No. 20	340	143.0	173	1.673	0.027	1.63
No. 21	340	146.1	187	1.751	0.032	1.79
No. 71	370	186.0	186	1.687	0.051	3.01
No. 72	240	218.3	199	1.794	0.021	1.16
Average females		173.4	186	1.726	0.033	1.89

d. The sense organs become dulled after prolonged defective feeding—the animals respond but slowly to stimulations of sound, or light or smell.

e. Defectively fed animals fail to breed.

3. The effect of defective feeding on the brain and olfactory bulbs is less than upon the rest of the body, but is, nevertheless, very marked. The olfactory bulbs are stunted and to a con-

siderably greater degree than is the entire brain. When defective feeding is begun in rats about thirty days of age, the bulbs of rats thus experimentally stunted form about the same percentage of the total brain weight as do the bulbs of rats of the same litters killed at the beginning of the experiment. Whereas, under normal conditions, the bulbs of older rats (up to one hundred and fifty days) are considerably heavier in proportion than those of the young animals. With prolonged defective feeding the proportional weight of the bulbs tends to become slightly greater.

4. Sick animals, especially those with lung infection, show a marked diminution in the relative weight of the olfactory bulbs, accompanied by a certain amount of loss in total brain weight.

III. EXERCISE EXPERIMENTS

1. *Previous experiments on the effect of exercise upon the albino rat*

Several investigators have worked upon problems connected with the changes in the albino rat occasioned by an increased amount of exercise. J. R. Slonaker in 1907 published observations upon four rats of different ages kept in revolving cages for a short period. In 1912, the same author published an account of further experiments along the same line, and although this time, also, the work was with a small group of rats, yet the experiment was continued during the natural life of the animals. Slonaker was working chiefly upon the problem of normal activity in its relation to age and sex but, incidentally, he made some few observations upon the comparative development of 'exercised' and normal rats. He found that "exercised rats are more active, more alert, and brighter in appearance than the control ones," but that "the control males reach their maximum weight at an earlier age than exercised males, and also greatly excel them" and that "control rats live longer than exercised rats." No observations were made on the effect of exercise upon any of the internal organs.

Donaldson, in 1911, conducted a series of experiments to ascertain the effect of exercise upon the central nervous system of the albino rat, using the same sort of apparatus—a revolving cage with cyclometer attachment—employed by Slonaker. He found that there was a slight increase in brain weight (2.4 to 2.7 per cent) to be attributed to the effect of exercise. This was what was to be expected in view of the heavier brain to be found in the wild Norway rat. The cord showed no effect. The olfactory bulbs were not weighed separately.

Hatai ('15) published a series of observations based upon his own experiments and upon those of the present writer, showing the rather marked effect of the same exercise conditions upon the weight of the internal organs. In these experiments, the brains of the test animals showed an excess of 4 per cent over the controls with no effect upon the cord.

2. Description of Experiments. Series D and E

As it had thus been demonstrated that the brain of the albino rat could be modified by exercise in the revolving cage, it remained to determine whether, under such conditions, the olfactory bulbs would show a more marked variation than the brain as a whole.

For this work, also, large litters of stock albinos, were chosen. Each litter was weaned and divided into three groups when about thirty-five days old. One group constituted the 'Initial Controls,' and these were killed and examined as in the previous experiments. The second lot, the 'Final Controls,' was set aside in cages under the normal living conditions of the colony. The third group was used for the experiment. Each of these test animals was placed by itself in a wire revolving cage such as had been used by Slonaker, and later by Donaldson and Hatai. Each cage was 5 feet in circumference with an open nest box fastened to the central fixed axis. From this axis the food was suspended so that, theoretically, the rat must descend to the floor of the cage to eat. Practically, some rats soon learned to avoid this and so escaped a considerable amount of enforced exercise.

Each cage was provided with a cyclometer. Readings were made and recorded six times a week. These cyclometer readings showed only the activity of the rats when the cage revolved and were therefore incomplete, since some rats learned to play from side to side of the cage and keep it from revolving, while others learned to run up the middle of the sides in such a way as to hold the cage at rest. But most of the rats soon learned to run the cages and appeared to enjoy it.

The rats were fed on the same diet as the controls and all the animals were weighed at intervals of about two weeks.

3. *Series D. Rats in revolving cages for thirty days*

There were but two litters in this series. One litter was weaned and set aside at thirty-five days of age and the other at forty days. Both litters were subjected to exercise in the revolving cages for a period of only thirty days. All were killed at the end of the thirty days of exercise.

a. Results. The exercised males of these two litters gained more rapidly in both weight and body length than did the controls, while the females fell behind. The superior growth of the test males was sufficient to bring the averages for both males and females up to 113 per cent of the weight of the controls and to 104 per cent of the length (tables 13 and 14).

The records of the activity of Series D were accidentally destroyed, but as these were for a period of but thirty days, they would be of little value save in adding further evidence that the female rat becomes active sooner than the male.

While, on the average, there is no difference in the absolute brain weight of the test rats in Series D from that of the controls, when both are compared with the reference table values in The Rat (Donaldson, '15), according to the method there suggested (pp. 4 and 5), yet I believe the bulbs do show, even after this short period, some effect of the unusal activity (tables 13 and 14). In the females, the bulbs make up 4.46 per cent of the brain weight in test rats as compared with 4.36 per cent in the controls. With the males, the difference was more marked—4.55 per cent in tests to 4.20 per cent in controls, making a joint average for males and females of 4.51 per cent in tests against 4.32 per cent

TABLE 13. SERIES D

Test animals

Albino rats kept in revolving cages for thirty-three days after weaning

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
PR$_1$	70	88	148	1.489	0.019[1]	1.30
T$_3$	68	131.7	170	1.871	0.079	4.21
T$_2$	68	140.9	176	1.831	0.085	4.63
PR$_3$	70	173.2	185	1.784	0.085	4.79
Average males		148.6	177	1.829	0.083	4.55
Females						
T$_1$	68	102.9	158	1.744	0.079	4.53
PR$_2$	70	116.0	162	1.653	0.073	4.39
Average females		109.5	160	1.698	0.076	4.46
Average males and females		132.9	170	1.776	0.080	4.51

[1] Lungs infected. Rat undersized in every way, therefore not included in averages (Series C, Sick rats, p. 218).

in controls, the olfactory bulbs of the former being, therefore, 7 per cent heavier than those of the latter.

4. Series E. Rats in revolving cages for ninety-eight to one hundred and three days

The test animals of this group were kept in the revolving cages for fifteen weeks. At the end of that time, three pairs of test animals and one pair of controls were mated (brother to sister in each case). Some digestive trouble appeared in the cages of control rats rather early in the experiment and most of the rats died, while the remaining animals failed to attain a normal growth, so that satisfactory final controls were lacking for this group. But the rest of the test animals and the surviving controls were killed at the end of the fifteen weeks, measured,

TABLE 14. SERIES D

Final controls

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
PR_5	70	100.7	154	1.636	0.061	3.72
PR_6	70	90.0	155	1.538	Bulbs unequal[1]	
T_4	68	132.4	170	1.802	0.084	4.64
Average males		116.6	162	1.719	0.072	4.20
Females						
T_5	68	110.8	159	1.780	0.081	4.52
T_6	68	115.6	165	1.774	0.077	4.32
PR_7	70	130.5	170	1.704	0.074	4.32
Average females		119.0	165	1.753	0.077	4.39
Average males and females		118.0	164	1.739	0.075	4.32
$\frac{\text{Test}}{\text{Control}}$			103.7%	102.1%	106.8%	

[1] Lungs slightly infected. Not included in average.

weighed, examined, and bulbs preserved exactly as in the underfeeding experiments.

The mated animals were kept about one hundred days longer to see whether the exercise of the previous weeks would show any effect upon fertility.

a. Results. General body growth. We find by examination of the records of body weight taken at two-week intervals during the experiment, that up to the time the larger set of control rats fell sick, the exercised animals were gaining less rapidly in weight than were the controls. From the time of the illness, some five weeks after the beginning of the experiment, the control rats fell off in weight, and with a single exception, they never recovered. Litter W escaped the infection and the weight records for the six rats composing it are as follows:

TABLE 15

Record for Litter W, Series E, showing gain in weight for individuals in revolving cages and for controls

	TEST RATS			CONTROL RATS		
	W_6 (m)	W_4 (f)	W_1 (f)	W_2 (m)	W_3 (m)	W_5 (f)
Initial weight	44.0 g.	40.5 g.	50.5 g.	43.5 g.	50.0 g.	40.5 g.
2 weeks weight	60.4	62.0	65.6	63.2	68.7	69.2
4 weeks weight	97.0	82.0	103.0	107.0	120.0	89.0
6 weeks weight	139.0	124.0	142.2	139.0	148.8	119.0
9 weeks weight	186.0	142.0	170.5	194.0	212.0	148.0
30 weeks weight	210.0	148.0	187.0	205.0	224.0	150.0
Final length	209 mm.	182 mm.	198 mm.	207 mm.	200 mm.	190 mm.

The test rats from Litter W were, on the whole, slightly longer and lighter in weight than the control animals. The majority of individuals in Litter W proved to have abnormal brains—one or both olfactory bulbs being very much undersized. The brains, therefore, could not be used for comparison and the litter was excluded from the tables. For comparison with the rest of the litters of Series E, it was necessary to use other stock litters, as will be described later (tables 18 to 25). The comparisons are not, therefore, of as much value as they would be were the controls from the same litter. On the average we find body length slightly more, and body weight slightly less, in test animals (table 25). I think we may conclude that these results agree in general with those of previous investigators indicating that exercise has but a slight effect, if any, upon either body weight or body length.

The size of the viscera was considerably modified. These results have been incorporated in the report by Hatai ('15).

Activity of exercised animals. These rats showed great individual difference in the amount of activity and in the age at which they became most active (tables 16, 19, 21, 25). In these respects, there was also a considerable difference in litters as shown by the following record.

If we take the record of these same rats for ninety-three days we get an average of 5.76 miles per day for males, and 5.96 miles

TABLE 16

Activity record of rats in revolving cages for one hundred and three days. Series E

ANIMAL	TOTAL MILES	MILES PER DAY	ANIMAL	TOTAL MILES	MILES PER DAY
Y_7M	914.5	8.9	Y_1M	559.7	5.4
Y_8F	770.5	7.5	Z_3M	476.3	4.6
Y_6F	724.0	7.0	X_6M	470.8	4.6
Y_4F	705.3	6.8	X_2F	458.7	4.5
Y_2M	689.0	6.7	Z_6F	457.1	4.4
Z_5M	577.8	5.6	Z_4F	446.6	4.3
Average for males	614.7	5.96			
Average for females	593.7	5.76			

for females, and if we go back still further we get a still higher average for the females and lower for the males. The males were slow to begin to run the cages. An extreme example, Y_1 of the present series, ran less than 2 miles during the first five weeks in the cage, but became extremely active during the last four or five weeks making a final average of 5.4 miles per day, a record almost equal to the average for the entire lot of males. The females soon learned to run the cages and became very active at an early age. During the last weeks of the experiment, the activity of practically every female in the series was on the decline. I think from a study of all the records it may be concluded that while, in the revolving-cages, the females reach the period of greatest activity earlier than do the males, yet in the long run, the records of a large number of males and females would average about the same.

Possible effect on fertility. There is some indication that the fertility of the albino rat is increased by exercise. In the cases of the three pairs of exercised rats which were mated, the following record of offspring was obtained, together with the record of one control pair.

The average size of litter for normal stock albinos has been found to be between 6 and 7 individuals (Donaldson '15). This is about the average for the control pair, while the averages for the three test pairs is considerably higher—13, 10.5, and 9.

TABLE 17

TEST PAIRS		CONTROL PAIR	
W_1	21st day after mating, 12 young	W_8	24 days after mating, 3 young
and	61st day after mating, 11 young	and	50 days after mating, 12 young
W_6	102d day after mating, 16 young pregnant	W_2	102 days after mating, 0 young not pregnant
Y_6	22d day after mating, 9 young		
and	67th day after mating, 9 young		
Y_3	102d day after mating, 0 young not pregnant		
Z_4	22d day after mating, 12 young		
and	88th day after mating, 9 young		
Z_3	102d day after mating, 0 young not pregnant		

It is significant also that the pair making the record of an average of 13 per litter for three successive litters, and the control pair are from the same original litter. Of course the numbers here are too few to enable one to draw conclusions but it would not be surprising to find some correlation between the greater weight of the sex organs in the exercised rats (Hatai '15) and the fertility of these animals.

Effect on brain and olfactory bulbs. It has already been noted that most of the control rats of this series were lost through disease. For comparison with the exercised rats, a set of controls used in Series A of the defective feeding experiment was chosen (table 20). These rats seemed better suited for the purpose than any others because they had been born at the same season as the test animals and reared in the same laboratory, so the food from day to day was the same for the two sets of rats. Among these, it was possible to find records of eight rats of almost the same body length and weight and of approximately the same age as the exercised rats killed at the end of the experiment. For the four which were mated and not killed until they were two hundred and thirty-eight days old, it was not possible to get controls of the same age, the four oldest of the controls (table 22) averaging only one hundred and sixty-nine days and the body length being 6 per cent less than that of the test animals. But as these are beyond the one hundred and fifty day limit, up to which time the bulbs increase in relative weight, the difference is not so serious a matter as it would be were the rats younger.

The set of test animals killed at the end of one hundred and three days of exercise, gave bulbs averaging for the males 4.28 per cent of the entire brain weight, and 4.60 per cent for the females—an average of 4.41 per cent for the entire set (table 19). When these results are compared with the controls (table 20) we find that while the test animals were 1 per cent shorter than the controls and had brains 2 per cent lighter in weight, the olfactory bulbs were 3 per cent heavier. These results seem to indicate that the olfactory bulbs of the test animals have been affected by exercise.

An examination of the records for the initial controls of the litters concerned seems to give additional weight to this supposition. See table 23 below.

TABLE 18. SERIES E

Initial control animals

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
W_1	30	26	99	1.338	0.052	3.89
Y_{10}	30	36	104	1.254	0.052	4.18
W_7	30	41	109	1.472	0.056	3.83
Z_8	30	41	112	1.444	0.050	3.46
Z_7	30	46	121	1.452	0.056	3.87
Average males		38	109	1.392	0.053	3.84

Females

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
Y_8	30	29	96	1.21	0.043	3.57
X_5	30	24	96	1.280	0.045	3.52
X_1	30	28	101	1.338	0.034	2.51
W_6	30	34	107	1.335	0.043	3.22
W_5	30	43	114	1.432	0.048	3.34
Average females		31	103	1.319	0.043	3.22
Average males and females		35	106	1.356	0.048	3.54

TABLE 19. SERIES E

Test animals

Albino rats kept in revolving cages for one hundred and three days after weaning

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT	AVERAGE NUMBER MILES PER DAY
	days	*grams*	*mm.*	*grams*	*grams*		
X_6	134	233	193	1.927	0.079	4.12	4.6
Y_1	135	188	195	1.693	0.070	4.16	5.4
Z_5	135	226	198	1.849	0.080	4.30	5.6
Y_7	135	237	205	1.875	0.085	4.54	8.9
Average males		221	198	1.836	0.079	4.28	6.1

Females

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT	AVERAGE NUMBER MILES PER DAY
X_3	134	151	177	1.661	unequal		4.5
Y_8	135	157	181	1.655	0.081	4.80	7.5
Z_6	135	162	180	1.796	0.078	4.32	4.4
Y_4	135	169	191	1.693	0.079	4.64	6.8
Averages females		162	184	1.715	0.079	4.60	5.8
Average males and females		196	192	1.784	0.078	4.41	5.95

As we see, the brains of the initial controls for the test animals (X, Y, Z) averaged but 91 per cent of the weight of the initial controls for the final controls (L, N, O, T, U, V); the olfactory bulbs but 89 per cent. Since it has been found that brain and olfactory bulb weight are pretty uniform for any given litter, and that when we find light or heavy brains or bulbs in the initial controls, we are fairly sure of finding the same relative development in the adult animals of the same litters, it seems fair to assume that normal adult individuals of litters X, Y, Z, would have had relatively lighter brains and bulbs than were found in adults of litters L, N, O, T, U, and V. If this assumed relation were true, then the results given in tables 19 and 20 doubtless would fall into line with those of previous experiments in which exercised rats showed an increase in brain weight over the

TABLE 20. SERIES E

Final control animals[1]

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
T_6	160	223	202	1.890	0.085	4.51
U_3	146	228	202	1.987	0.081	4.06
N_3	121	203	205	1.825	0.072	3.93
L_6	157	274	218	2.021	0.077	3.81
Average males		232	206	1.931	0.079	4.08
Females						
H_3	93	127	172	1.559	0.063	4.03
V_7	124	144	177	1.672	0.067	4.03
T_7	213	183	187	1.801	0.082	4.56
O_1	115	175	188	1.792	0.083	4.62
Average females		157	181	1.706	0.074	4.32
Average males and females		195	194	1.818	0.076	4.20
Series A $\frac{\text{Test}}{\text{Control}}$			99%	98%	103%	

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[1] Data from stock Albinos used for controls in Defective Feeding Series A and again used for comparison here, since the original controls died early in the experiment.

controls, and would indicate an even greater gain in bulb weight for the test animals than is indicated in the tables.

In the same way, we may compare the initial controls for the mated test animals and those for Series A used for a standard (tables 21 and 22). We find the initial relations practically the same as for the group just discusesd.

In the final results (tables 21 and 22) we see that although the test rats were older, with bodies 6 per cent longer, the brains were actually 5 per cent lighter in weight. Here again, examination of the initial controls suggests that in all probability there was not an actual loss of brain weight in the exercised animals.

TABLE 21. SERIES E

Test animals

Albino rats kept in revolving cage for one hundred and three days after weaning. At end of that time mated and allowed to rear 2–3 litters. Age, when killed, about eight months.

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS PER CENT BRAIN WEIGHT	AVERAGE NUMBER MILES PER DAY
Males							
	days	*grams*	*mm.*	*grams*	*grams*		
Z_3	238	299	221	2.018	0.092	4.54	4.6
Y_3	238	311	228	1.842	0.094	5.10	6.7
Average males		305	225	1.930	0.093	4.81	5.7
Females							
Z_4	238	216	202	1.777	0.080	4.58	4.3
Y_6	238	156	203	1.654	0.080	4.81	7.0
Average females		186	203	1.716	0.080	4,66	5.7
Average males and females		246	214	1.823	0.086	4.74	5.7

TABLE 22. SERIES E

Control animals

Stock albinos used for control in defective feeding experiment, Series A. The four oldest of this set chosen for present tests since original controls died early in the experiment.

AVERAGE	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS PER CENT OF BRAIN WEIGHT
U_3	146	228	202	1.987	0.081	4.06
L_6	157	274	218	2.021	0.077	3.81
T_6	160	223	202	1.890	0.085	4.51
T_7	213	183	187	1.801	0.082	4.56
Average		227	202	1.925	0.081	4.23
Series A $\frac{\text{Test}}{\text{Control}}$			106%	95%	106%	

TABLE 23

INITIAL CONTROLS FOR TEST ANIMALS				INITIAL CONTROLS FOR CONTROL ANIMALS. (DEFECTIVE FEEDING EXPERIMENT)			
Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight	Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight
	grams	*grams*			*grams*	*grams*	
X, Y, Z	1.305	0.047	3.60	L, N, O, T, U, V.	1.431	0.058	3.69
$\frac{\text{Test}}{\text{Control}}$	91%	89%					

TABLE 24

INITIAL CONTROLS FOR TEST ANIMALS				INITIAL CONTROLS FOR CONTROL ANIMALS (DEFECTIVE FEEDING EXPERIMENT)			
Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight	Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight
	grams	*grams*			*grams*	*grams*	
X and Y	1.340	0.051	3.76	L, U and T	1.458	0.055	3.76
$\frac{\text{Test}}{\text{Control}}$	92%	92%					

But, be this as it may, we find the bulbs of these test animals actually 6 per cent heavier than those of the controls, the bulbs making 4.74 per cent of the total brain weight, while those of Series A controls were only 4.23 per cent of the total weight of the brain.

Since we have no true control series for comparison, we can not, of course, draw conclusions as to the absolute gain in brain weight after exercise. But of the gain in olfactory bulb weight in exercised animals, there seems to be no doubt.

When we turn to table 25 and note that the average percentage weight for the bulbs in case of 29 normal rats (59 to 83 days old) is 4 per cent, while a study of table 13 shows there was no rat there recorded (save one sick one) in which the per cent fell below 4.21 per cent, while the average was 4.51 per cent, we must be convinced, I believe, of the reality of the effect of exercise. For the older rats, likewise, when we compare tables 19 and 25,

TABLE 25

(Summary) Showing comparative development of the olfactory bulb

Stunted series

TABLE	NO.	HISTORY	AGE	BODY WEIGHT	BODY LENGTH	AVERAGES FOR BRAIN WEIGHT	BULBS WEIGHT	BULBS PER CENT BRAIN WEIGHT	RATIO AVERAGE TEST TO AVERAGE CONTROL IN			AVERAGE MILES PER DAY
									Body length	Brain weight	Bulbs weight	
			days	*grams*	*mm.*	*grams*	*grams*		*per cent*	*per cent*	*per cent*	
II and XVIII.....	38	Initial control. Underfed and Exercise Experiment	3; 18–20 35; 29–34	40	111	1.400	0.050	3.54				
X.............	9	Underfed from birth	27–53	18	83	1.091	0.034	3.14				
XI.............	3	Underfed from birth	77	31	102	1.437	0.050	3.48				
III.............	26	Underfed 29–32 days	59–62	57	126	1.504	0.053	3.52	77	91	81	
IV.............	26	Controls for above	59–62	119	164	1.651	0.066	3.99				
V.............	3	Underfed 49 days	78–83	44	124	1.487	0.050	3.39	69	87	70	
VI.............	3	Controls for above	78–83	154	180	1.719	0.072	4.16				
VII.............	13	Underfed 59–130 days	93–160	51	122	1.524	0.058	3.33	63	85	77	
VIII.............	12	Controls for above	93–160	187	193	1.772	0.076	4.30				
		Sick animals										
XII.............	3	Bowel infection	134–135	146	176	1.647	0.050	3.02				
XII.............	8	Infected lungs	70–370	187	190	1.742	0.034	1.96				
		Exercise series										
XIII.............	6	Revolving cage 30 days	68–70	133	170	1.776	0.080	4.51	103.7	102.1	106.8	
XIV.............	6	Controls for above	68–70	118	164	1.739	0.075	4.32				
XIX.............	8	Revolving cage 103 days	134–135	196	192	1.784	0.079	4.41	99	98	103	5.95
XX.............	8	Controls for above	93–213 (av. 141)	195	194	1.818	0.076	4.20				
XXI.............	4	Revolving cage 103 days and mating	237	202	214	1.823	0.086	4.74	106	95	106	5.65
XXII.............	4	Controls	146–213 (av. 169)	227	202	1.925	0.081	4.23				

we see that the average for 12 controls (90 to 160 days old) was 4.26 per cent while only two test animals fell as low as this (one of these was of abnormally light body and brain), and the averages were 4.41 per cent and 4.74 per cent for four and one-half months and eight months respectively.

5. Summary

1. The results of the present experiments agree with those of previous investigators in that they show no marked effect of exercise either upon body length or body weight in the albino rat.
2. The female albino becomes very active earlier than does the male but the activity of the male later increases to such an extent that the total activity for the two sexes for long periods is probably about equal.
3. These experiments suggest that there is an increase in fertility correlated with increase in the size of the reproductive organs.
4. The brain weight is slightly increased by exercise.
5. The weight of the olfactory bulbs of albino rats exercised in revolving-cages for periods of from thirty to one hundred days, is considerably increased. The bulbs of such rats form from 4.41 to 4.74 per cent of the total brain weight as compared with 4.20 to 4.32 per cent in rats reared under normal colony conditions. These bulbs show an increase of 5 to 11 per cent over and above the increase in weight manifested by the entire brain.

IV. CONCLUSIONS

From the preceding observations we may conclude that we are able to modify the olfactory bulbs of the rat by changing the conditions under which it lives and to modify them to a considerably greater degree than we can change the rest of the brain. In cases of stunting, the bulbs tend to overcome the effect, to a certain extent, as time goes on. With exercise the effect seems to increase with age. Yet the bulbs respond more markedly to the stunting effect of defective feeding or sickness than to the stimulating effect of exercise.

A histological study of these modified bulbs will be presented in the second part of this paper.

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PART II. ON THE NUMBER OF NERVE CELLS IN LARGE AND SMALL OLFACTORY BULBS

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I. INTRODUCTION

The foregoing studies have shown that it is possible to change the relative weight of the olfactory bulbs in the albino rat (Holt, '17). This relative weight is decreased by a defective diet and increased by exercise. Such being the case, it seemed very desirable to make a histological comparison of the bulbs which had been stunted by a defective diet or enlarged by exercise, with those of rats reared under normal colony conditions. For this purpose it was of course essential to find a method of fixation and treatment which would give uniform results. Fixation in Ohlmacher's solution as recommended by King ('10) for the study of cortex cells, gave very satisfactory results, but her statement that "various individuals react differently although subjected to the same course of treatment," and her tables (loc. cit., p. 231) showing a variation in shrinkage ranging from 2 to 18 per cent in brains so fixed, suggested that it would be best to examine this method a little more in detail. Unless uniform results could be obtained by it, this method would, of course, be un-suited to comparative study of the size of bulb elements. Accordingly, the method was further tested, and at the same time an examination was made of the effect of Müller's fluid and of Orth's Formol-Müller solution upon the various parts of the brain.

A long series of experiments demonstrated quite conclusively the following points which have an important bearing upon the present investigation.

1. Of the three fixing fluids tested, Ohlmacher's solution causes the least change in weight in brain tissue.
2. Orth's solution (cold) causes a slight increase in weight.
3. Müller's solution causes a very considerable increase in the weight of brain tissue as has already been noted (Donaldson, '94).
4. Olfactory bulbs, fixed in Ohlmacher's solution, reach a state of equilibrium at the end of twenty-four hours; fixed in Formol-Müller, they reach this state at about the end of one week; fixed in Müller's solution alone, changes continue from six weeks to two months.
5. There seems to be no appreciable individual variation in the reactions of albino rat brains of like age to Ohlmacher's solution, to Müller's fluid, or to the Formol-Müller solution. The results obtained by Dr. King are due apparently to the fact that the brains which were weighed in her experiments had been fixed for varying short lengths of time and the initial changes in weight were so rapid that there appeared to be a considerable difference in the way the various brains reacted to the fixative, when in reality, had all the brains been fixed for exactly the same length of time, no such large disagreement would have been found.

II. THE PROBLEM OF SIZE DIFFERENCES

Although under normal conditions, there is a good deal of variation in the size of the olfactory bulb of the albino rat, we have found that it is possible, experimentally, to increase this range of variation to a very considerable degree. The question next arises as to the structural cause of the difference in size. Is it one of size of elements or of their number? Have we more cells and fibers in the heavier bulb or are the cells and fibers merely of a larger size? The present paper deals only with the question of cells. The fibers have yet to be examined.

III. TECHNIQUE AND METHODS OF STUDY

Since experiments on the effect upon the rat brain, of Ohlmacher's, Müller's, and the Formol-Müller solutions have demonstrated that for brains of like ages there is a definite, practically unvaried, swelling or shrinking reaction for any given fluid, the brains of the rats used in the defective diet and exercise experiments were fixed in these several solutions for histological comparison. For the present cell study the method adopted was that recommended by King ('10) for study of the cortex, namely fixation in Ohlmacher's solution for twenty-four hours, followed by one hour in 85 per cent alcohol, three to four days in iodized 70 per cent alcohol, double embedding in celloidin and paraffin, and staining in carbol-thionin and eosin. However, after the first trials, this method was varied in the matter of embedding. For such small objects as the olfactory bulbs, paraffin proved more satisfactory when used alone. Sections were cut 8μ thick and mounted serially. A rather deep thionin stain gave the best results for cell enumeration.

1. Preparation of sections

At first, some bulbs were cut sagittally and the largest sections compared. In the study of these sections the number of cells in the gray layer of the different bulbs was found to be so nearly identical that it was decided to attempt a thorough study of cell number.

In dissecting a rat brain into its parts, the bulbs are cut from the brain in such a way as to leave an appreciable portion of the bulb attached to the cerebrum. The method followed was to place the brain, ventral side down, on a flat surface and with a knife held in a plane perpendicular to the table, to sever the bulb at the point where it disappears beneath the cerebrum (plate 1).

This is the part of the bulb which is weighed, and since all bulbs are removed in the same way, it has been assumed that we have corresponding portions for comparison. Because the recorded weights represented only this portion of the bulbs, it seemed advisable at first to compare the cell elements of these parts only. Accordingly cross sections of the bulbs were made but unfortunately, as appeared later,most of the test series were not complete for the portion of the gray substance beneath the hemispheres. The method of meeting this difficulty will be described later.

2. *Methods of study*

The study of sections was made largely with the aid of the Edinger projection apparatus. Cell counts were made by projecting the sections onto white wrapping paper, outlining the area, and punching the image of each cell nucleus with a tallying register fitted with a sharp prong in place of the usual blunt register arm (Hardesty '99). The hole punched by this prong insured against counting the same cell twice. It also left a permanent record of any particular region which could be reexamined later. In some cases the count for each section was recorded; in others, the whole number of sections to be counted were registered consecutively and no record made until the end. Occasionally a section was recounted—to serve as a check on the work.

IV. GENERAL DIFFERENCES IN SIZE

Most of the comparisons of size and the determinations of cell number have been made on bulbs stunted by a defective diet, and on their respective controls. Only two bulbs of the exercise series have yet been examined.

The general differences in size between young and mature, stunted and normal olfactory bulbs are very well illustrated by the sections shown in Plates 1, 2, and 3. Figure 1 of plate 1 is a camera drawing of a median sagittal section through the bulb of a rat stunted by feeding for thirty-one days on a corn diet. The body length was 138 mm.; brain weight, 1.547 grams and the weight of the corresponding bulb which was removed and weighed, was 0.020 gram—the approximate weight then of the bulb shown in the drawing. Figure 2, plate 1, is a median sagittal section through the control bulb. The rat from which this was taken was 166 mm. long, with brain weight of 1.698 grams, and the weight of the corresponding bulb was 0.032 gram. Like the bulbs of very young rats, the gray layer of the stunted bulbs extends somewhat further, in proportion, beneath the cerebrum than in the case of normal older individuals.

When the stunted bulb is compared with its control, there appears to be a rather uniform size difference involving all parts of the bulb. It is hard to compare the outer fiber layers owing to the difficulty in removing the bulbs perfectly from the skull. The anterior end of the fiber layer is very likely to be entirely torn away and sometimes the ventral side also suffers. However, it is plain that the glomeruli of the larger bulb are much larger and more open; the granular cells are not so closely packed together; and the gray layer is usually broader in the larger bulb and the inner granular area considerably more extensive. These differences between the peripheral portions are illustrated by the more highly magnified mid-dorsal areas *S* and *S* of figures 1 and 2, shown in figures 3 and 4 of plate 2.

Plate 3 shows three cross sections; through Q_5, a thirty-day control bulb (fig. 5); M_4, a sixty-two-day stunted bulb (fig. 6); and M_5, the sixty-two-day normal control (fig. 7), for M_4. These sections were made through the region where the bulbs are usually cut from the brain. The figures illustrate another typical difference. The normal bulb (figs. 5 and 7), as it grows, elongates more rapidly than it increases in thickness and it tends to grow faster dorso-ventrally rather than laterally. In these figures, the outer fiber layer is probably complete at the sides. Ventrally it has doubtless been torn away to some extent in all three bulbs. The difference in size of the glomeruli is well shown here, but while there is a greater area of gray matter in figure 7 than in the other two, the gray layer seems narrower than in M_4 (fig. 6). The companion bulb of Q_5, (fig. 5) weighed 0.024 gram, that of M_4 (fig. 6), 0.025 gram while M_5 weighed 0.037 gram (fig. 7). The portion of Q_5 anterior to the section illustrated, was about 1350μ long, while M_4 had 1500μ anterior to the section, and M_5, 2000μ. The differences in size are confined to no one region but are distributed somewhat proportionally through the different layers.

V. COMPARISON OF CELLS OF GRAY LAYER

1. *Size and number of small cells in molecular layer*

It has been the general impression that, within certain limits, the size and weight of the brain are indices to its functional capacity. In the phylogenetic series, of course, it is, with one or two exceptions, true that increase in intelligence is accompanied by increase in the relative size of the brain. So within any given species of mammals, it has been assumed that the more efficient brain is the larger and heavier.

The question as to whether, within such a group, increase in size of the brain means an increase in the number of nerve elements or in the size of the elements themselves, becomes an important one. For an increase in the number of elements should give increased functional possibilities. So, if we find in comparing large and small brains or divisions of brains from closely related animals, that the larger structure contains a greater number of cells and fibers, then we have reason to expect from the larger and more complex structure the greater degree of efficiency.

If, on the other hand, the number of elements is found to be uniform for the part under consideration, then we must conclude that the large and the small brains have potentially the same ability to function, save as their efficiency may be affected by the size or degree of development of the individual elements.

The small cells of the molecular layer (*mo*, fig. 2) show more cytoplasm; or perhaps we may say that it is possible to distinguish more cells with cytoplasm in the molecular layer of large bulbs than of small ones. For example, the section of F_1 shown in figure 1 shows 68 cells between mitral layer and glomeruli, in which cytoplasm may be distinguished, while the control, F_5, shows 158 such cells. Corresponding sections through M_1, a thirty-day control, and C_3, a sixty-day underfed bulb, show 108 and 103 cells with cytoplasm.

Although a difference in cell size appeared, there seemed to be little difference in numbers of cell elements in the gray layer. Although it is not always possible to distinguish between the nuclei of very small cells and possible cross sections of fibers under the conditions used for counting—yet the error due to this difficulty is probably negligible. A preliminary count was made of all elements, having the appearance of nuclei in the largest sections of the bulbs F_1, C_3, F_5, F_6, and M_1, with the following results.

TABLE 1

INITIAL CONTROL				TEST				FINAL CONTROL			
Bulb	Age	Bulb weight	Number cells	Bulb	Age	Bulb weight	Number cells	Bulb	Age	Bulb weight	Number cells
	days	*grams*			*days*	*grams*			*days*	*grams*	
M_1....	30	0.029	2172	F_1	62	0.020	2569	F_5	61	0.032	2569
				C_3	59	0.021	2604	F_6	61	0.033	2693

These counts for the test and final control bulbs suggested so strongly that the number of cells is the same for bulbs of different sizes that attention was turned entirely to the investigation of this point. At first longitudinal sections were used, but these were soon abandoned for two reasons. First, it seemed desirable to be able to count the cells of just that portion of the bulbs corresponding to the part weighed; and second, the longitudinal sections presented so many irregularities that it was necessary to count many more sections to approximate the true average than in the case of the cross sections. Counts were made of all elements in the gray layer outside the mitral layer between the tip of the bulb and the point at the proximal end where the gray layer is first interrupted on the dorsal aspect of the bulb (see figs. 5, 6, 7). These counts consumed a vast amount of time and when completed seemed to disagree with the observations already made upon the longitudinal sections (table 2).

A first glance at the table would indicate that the small bulb has fewer cells and would suggest that this difference in cell

TABLE 2

BULB	AGE	HISTORY	BRAIN WEIGHT	WEIGHT 1 BULB	NUMBER SMALL CELLS
	days		*grams*	*grams*	
X_1....	30	Control	1.338	0.017	636,656
M_4....	62	Underfed 31 days	1.461	0.025	662,982
G_{12}....	62	Underfed 31 days	1.543	0.027	601,982
G_6.....	61	Normal control	1.630	0.031	675,305
M_5....	62	Normal control	1.711	0.037	716,582
X_6....	134	Revolving cage 104 days	1.927	0.040	789,680

number is one of the factors in bulb size. But corresponding sagittal sections had given fairly close agreement in numbers and the study of sagittal sections made it more and more evident that these counts of cross sections could be taken only to compare the parts commonly considered the bulb and not for an enumeration of the cells in the whole bulb. The difference in shape in the large and small bulbs made it apparent that a true count must be made either from sagittal sections or from cross sections cut through the entire length of the gray matter covering the bulb. Comparison of such sections as figures 1 and 2 made it clear that if we had, in reality, a constant number of cells in the gray layer, the numbers in the regions here designated as the 'bulb' could scarcely be expected to show any closer agreement than we find in this table, and would probably have the relations there given. For the larger and better developed the bulb, the greater the proportion of it lying anterior to the cerebrum, while the young or the stunted bulb runs somewhat further back beneath the hemisphere and so some of the cells escaped enumeration. For example, M_5, a bulb of 0.037 gram, has 271 sections containing mitral cells in the portion of the bulb beneath the cerebrum. M_4, the test bulb of this litter, which weighed but 0.025 gram had 336 sections in this region. Taking these facts into consideration, the table in question pointed to a uniformity rather than variation in numbers corresponding to size. Later we shall see how, in the light of the study of the mitral layer, a part of this table can be shown to closely conform to this supposition that the number of cells in the entire gray layer is approximately constant for olfactory bulbs of different sizes.

2. *Size and number of mitral cells*

The cells of the mitral layer show a good deal of variation in size and shape and there is much difference in these respects in different regions of the same bulb. This makes the comparison of the size of the mitral cells in large and small bulbs rather difficult.

But if all the mitral cells of a section from a small bulb are drawn with a high magnification by means of camera lucida or projection apparatus and those cells arranged side by side with a series from a corresponding section of a large bulb, drawn to the same scale, it is possible to make a general comparison. In this way the mitral cells have been compared, and there is no doubt I think that the mitral cells of large bulbs are larger and better developed than those of small bulbs.

Details of technique and examination. It was sometimes quite difficult to determine whether a cell should be counted or not. For instance, when counting mitral cells it was hard to know at times whether a cell was a mitral cell or a brush cell, as many cells occur in the mitral layer which are exactly like those large cells occurring in the molecular layer but which lack the typical mitral form. On the other hand, typical mitral cells occur not infrequently out in the molecular layer or even among the granules on the inner edge of the glomerular layer. For this reason and in order that there might not be any unconscious influence in deciding whether cells should be counted, an attempt was made to vary the order of procedure for each successive count.

A rather complete count was made of the mitral cells of fourteen bulbs and of the small cells of the gray layer in four bulbs. Eight of these were cut longitudinally and six cut transversely.

The first series counted were those of X_1, Initial control, Series E and X_6, Test, Revolving Cage Series. In both series every other section was counted for the region anterior to the cerebrum—corresponding to the portions of these same bulbs in which the small cells of the gray layer had been counted. The result was 64,470 cells for X_1, a 0.017 gram. thirty-day control bulb. The number for X_6, whose weight was 0.040 gram, was 73,950. To see whether there were any virtue in making so thorough a count of cross sections, the total number was computed from a recount of every 10th section, excepting at the most anterior end where every cell was counted in every section, until the sections showed a single layer of mitral cells. By this method the number obtained for X_1 was 64,775 cells, making a difference of only 0.4 per cent. For X_6 the count was 73,324, which was 0.8 per cent smaller than the more exact count obtained by counting half the sections. These differences were so small as to make the more exhaustive count seem unnecessary. X_6 gave an almost complete series through the entire gray layer so the count was completed, giving for the entire bulb 80,114 cells. The count of the mitral cells in X_1 could not be completed as the series had been cut, unfortunately, with the idea of comparing only the parts of the bulbs whose weights we knew, and which, therefore, extended back but a short distance under the cerebrum. The cells of these few sections were, however, counted, giving a total of 71,914.

The number of mitral cells in G_6 was computed from absolute counts of anterior and posterior ends of the series and by counting every tenth section through the rest of the series. M_4, M_5 and Q_5 ran so evenly that here in the middle portion of each series, only every twentieth section was counted; on either side of this portion, every tenth section, and all cells of all sections at either end.

With the sagittal sections, the task was more difficult and the results, I believe, less reliable for this reason: toward the sides of the bulbs, especially the median side, the sagittal series may give tangential sections of the mitral layer so that a single section may yield a count of 1500 cells whereas a section two or three removed on either side might have but 300 or so mitral cells. It can be easily seen that if the section to be counted, happened to fall in such a region, or entirely skipped such a region, the count would be considerably modified. Some of the bulbs gave no trouble of this kind while others were hard to count for this reason. G_3 was an interesting example of the way this may work out. A count was first made of all cells at either end of the series and those in every tenth section through the middle portion. The result when computed was 95,993 mitral cells. Then the middle section of every ten was counted with a total result of 83,974 cells. Two other series were attempted but abandoned as the bulbs were so irregular that an accurate count would have required the enumeration of the cells of at least every alternate section. The other bulbs, except C_3 for which every fifth section was counted, were fairly regular so that the mitral layer offered no such complications. For these, the method of counting all cells at either end of the series and those of every tenth section through the median portion was followed. The sequence of counts was varied with each bulb, and the records kept in various ways and not infrequent recounts made. The recounts were surprisingly close to the original, for, as has been stated, it is not always easy to decide whether or not a cell should be counted, and in focusing as one counts, a granule lying below or above a portion of a mitral cell sometimes looks very like a nucleus, but the error due to this cause is probably too small to be considered.

Details of counts of the different bulbs, arranged in the order followed in table 3.

Bulb X_1, thirty day, initial control. Cross sections. Mitral cells counted in every section of anterior end back to the first section in which the mitral cells appeared in a single layer. From this point, counts were made for every tenth section back to the cerebrum. By computation, the total number of mitral cells was 64,775. By a recount in which the mitral cells of every other section were enumerated the computed number was 64,470 making a difference of only 0.4 per cent in the two counts. The series of sections for the region beneath the cerebrum was incomplete, the posterior portion not having been preserved. A count was made, however, of the sections which were present. This number added to the number already counted by the second method, brought the total up to 71,914 cells.

Bulb E_1, Test, defective diet series. Sixty-two days. Sagittal sections. Mitral cells counted in all sections at either end of the series and for every tenth section between.

Bulb C_3 Test, defective diet series. Fifty-nine days. Sagittal sections. Counts made as in E_1.

Bulb Q_5, thirty day, initial control. Defective diet series. Cross sections. Mitral cells counted in all sections at both ends of the series.

TABLE 3

Giving number of mitral cells in one olfactory bulb of the albino rat

Arranged according to bulb weight

RAT	AGE	BODY LENGTH	BRAIN WEIGHT	WEIGHT 1 BULB	NO. MITRAL CELLS	REMARKS
	days	*mm.*	*grams*	*grams*		
X_1 Control.....	30	101	1.338	0.017	71,914	Very incomplete. Up to point of union with cerebellum 64,470 cells, cross section
E_1 Test........	62	138	1.547	0.020	71,527	Sagittal section
C_3 Test........	59	142	1.482	0.022	79,165	Sagittal section
Q_5 Control.....	30	97	1.316	0.024	82,192	Probably about 300 more cells. Cross section
C_5 Test........	59	150	1.578	0.024	70,625	Sagittal section
M_4 Test........	62	109	1.461	0.025	76,611	Cross section
G_3 Test........	61	136	1.456	0.027	83,974	Sagittal section
O_5 Test........	115	121	1.556	0.029	71,663	Sagittal section
G_6 Control.....	61	166	1.630	0.031	81,638	Cross section
F_5 Control.....	61	166	1.698	0.032	71,468	Sagittal section
C_7 Control.....	62	174	1.709	0.036	79,839	Sagittal section
M_5 Control....	62	169	1.711	0.037	76,596	Cross section
X_6 R. C. Test..	134	193	1.927	0.040	80,114	Up to point of union with cerebrum 73,950 cells, cross section
O_1 Control.....	115	175	1.792	0.041	72,333	Sagittal section (X_1 omitted from average)
Average.....					76,749	

Standard deviation σ = 4564
Probable error of the mean ±855

Through the middle region only every twentieth section was counted as the sections were extremely uniform. Through the two regions between this middle portion and the ends in which all cells were counted the mitral cells for every tenth section were counted.

Bulb C_5, test, defective diet series. Fifty-nine days. Sagittal sections. Mitral cells counted for all sections at both ends of the series and every fifth section of the rest of the series.

Bulb M_4, test, defective diet series. Sixty-two days. Cross sections. Mitral cells were counted as in Q_5. Count was made also of all cell elements in every alternate section in the gray layer back to the anterior end of the cerebrum. Computation was made for entire series.

Bulb G_3, test, defective diet series. Sixty-one days. Sagittal sections. Mitral cells were counted for all sections at ends of series and for every tenth between. The computed result was 95,993. The middle sections between every tenth were then counted, giving a

TABLE 4

Giving number of mitral cells in one olfactory bulb of the albino rat

Arranged by litters

AGE	RAT	WEIGHT 1 BULB	NUMBER MITRAL CELLS	PERCENTAGE DIFFERENCE OF TEST	REMARKS
days		*grams*			
62	F_1 Test	0.020	71,527 (S.)	+ 0.08	
62	F_5 Control	0.032	71,468 (S.)		
115	O_5 Test	0.029	71,663 (S.)	− 0.9	
115	O_1 Control	0.041	72,333 (S.)		
62	M_4 Test	0.025	76,611 (C.)	+ 0.2	
62	M_5 Control	0.037	76,596 (C.)		
59	C_3 Test	0.022	79,165 (S.)	− 0.9 } Ave.	
59	C_5 Test	0.024	70,625 (S.)	−12.0 } −6.5	
62	C_7 Control	0.036	79,839 (S.)		
30	X_1 30 d. T.	0.017	71,914 (C.)		Very incomplete
134	X_6 R. C. T.	0.040	80,114 (C.)		Slightly incomplete (not in average)
61	G_3 Test	0.027	83,974 (S.)	+ 2.9	
61	G_6 Control	0.031	81,638 (C.)		
30	Q_5 30 d. C.	0.024	82,192 (C.)		Probably 300 more cells
Average per cent difference of test....				− 1.8	

(S.) = Sagittal section.
(C.) = Cross section.

count for every fifth section of this region. The computation then gave a total of 83,974. Two other bulbs of this litter were also cut in sagittal sections and an attempt was made to count the mitral cells but the bulbs were so irregular that it would have been necessary to count practically every section, so these counts were abandoned.

Bulb O_5, Test, defective diet series. One hundred and fifteen days. Sagittal section. Counts made as in E_1.

Bulb G_6, control, defective diet series. Sixty-one days. Cross section. Counts made as in E_1.

Bulb F_5, control, defective diet series. Sixty-one days. Cross section. Counts made as in E_1.

Bulb C_7, control, defective diet series. Sixty-two days. Sagittal sections. Counts as in E_1.

Bulb M_5, control, defective diet series. Sixty-two days. Cross sections. Counts as in M_4 and computation of all cell elements in gray layer made for entire series.

Bulb X_6, test, revolving-cage series. One hundred and thirty-four days. Cross sections. Mitral cells counted by both methods described for X_1. Also all cell elements of the gray layer computed for the entire series as in M_4 and M_5.

Bulb O_1, control, defective diet series. One hundred and fifteen days. Sagittal section. Mitral cells counted as in E_1.

Table 3 gives the results of the counts of the mitral cells of fourteen olfactory bulbs, arranged according to bulb weight. It is obvious that there is no correlation between bulb size and the number of the mitral cells or between age—within the limits taken—and number of cells. The numbers range from 70,625 to 83,974 with an average of 76,750 cells for 13 bulbs, X_1 being omitted from the average. I am inclined to think 83,974 cells is too high a count for G_3 and that still closer enumeration might yield a lower number. A recount was made for C_5 counting every fifth section, as this was a somewhat irregular bulb and it was thought that might account for the variation of this bulb from the rest of the litter. But the recount gave practically the original number.

Table 4 which is arranged by litters indicates a striking agreement between the members of the same litter. With the exception of litter C, in which C_5 falls 12 per cent below the control in number of mitral cells, there is extremely little difference between test and control bulbs of the same litter. So we find that whether the bulb has been stunted by a defective diet, or enlarged by exercise, the number of mitral cells is practically constant for any given litter. The factors which have brought about a change in size of the olfactory bulbs have failed to affect the number of mitral cells, at least in the gray layer. The test and control counts are, with one exception, extremely close. This is a fresh example of the similarity in structure among members of the same litter—a relation which is continually appearing in the study of this animal.

3. *Study of the small cells in the gray layer*

The sections of bulbs M_4, M_5, X_1, and X_6 are all series in which a count was made of the small cells of the gray layer in the anterior portion of the bulb as well as of the mitral cells. Assuming that the relation between the number of mitral cells in two given sections of a bulb would be the same as that between the small cells of the gray layer in these same regions, computation was made of the total number of small cells in the gray layer of M_4,

M_5, and X_6. Bulb X_1 was too incomplete to make such calculation possible. Let us take M_4. The small cells were counted back to section 4, 1/1. We have the total number of mitral cells and also the number of mitral cells back to section 4, 1/1. This gives us the data for computing the total number of small cells as follows:

Mitral cells of M_4 to section 4, 1/1 50,729
Total mitral cells 76,611
66 per cent of mitral cells in anterior portion.
Number small cells to section 4, 1/1 662,982

If this number equals 66 per cent of the total number, then the total number of small cells in the gray layer would be 1,004,518.

If we treat M_5 in the same way we have:
Mitral cells to section 3, 6/12 55,775
Total mitral cells 76,595
73 per cent of mitral cells in anterior portion.
Number small cells to section 3, 6/12 716,382
Then total number small cells 981,619

According to this computation the test bulb would have two per cent more cells than the control.

Now if we treat X_6 in like manner we have the following:
Mitral cells to section 5, 1/7 62,060
Total mitral cells 80,114
77 per cent of mitral cells in anterior portion.
Small cells to section 5, 1/7 789,680
Then total number of small cells 1,020.258

These total numbers are strikingly close. The number of bulbs is too small to warrant us in drawing general conclusions but I think the results certainly point to close agreement in number even of the small cell elements in the gray layer.

While there is a constant increase in the number of myelinated fibers correlated with age, as has been demonstrated by Greenman ('13) for the peroneal nerve, Boughton ('06) for the oculomotor, Hatai ('02) and ('03) for both dorsal and ventral roots of several spinal nerves, and Dunn ('12) for the ventral root of the second cervical nerve; there is very little evidence of any true increase in the number of cells in the central nervous system after the first few days after birth. Allen ('12) found dividing cells in the cerebellum up to twenty-five days and in the cerebrum up to twenty days, with a few along the lateral walls of the lateral ventricles until the end of the second year. Hatai observed an increase in number of cells in the spinal ganglia, corresponding to increase in age but this increase was attributed in part, at least, to failure to count all the ganglion cells in very small animals. Ranson ('06) in a study of the second cervical nerve found no correlation between the number of cells and the number of myelinated fibers, neither did he find the number of cells to vary with the age of the rat.

The results of the present investigation of the number of cells in the olfactory bulb help to confirm the impression that the number of cells in the central nervous system becomes fixed at an early age so that after the first three or four weeks at least, there is no material change in the cell number.

This study also gives us reason to believe that the number of small and of mitral cells in the gray layer of the olfactory bulb is very nearly the same for all individuals with especially close agreement between individuals of the same litter. It seems fairly evident that while external conditions may modify to a considerable extent the size of the brain of the albino rat and especially the size of the olfactory bulbs, the only effect is upon the relative development of the individual cells. The number of cells remains the same. The fibers have yet to be examined.

It is important to bear in mind in a determination of this sort—e.g., the number of mitral cells—that a fixed number, in the physical sense, is not to be expected, for all organisms are normally variable in all of their parts, variability being an essential character for living things; so the number which is obtained gives a mean value which we take to be characteristic for the species under the present conditions, but around which equally characteristic variations also occur.

VI. CONCLUSIONS

1. For bulbs of different ages and sizes, the regions anterior to the cerebrum, which are commonly considered the bulbs, are not strictly homologous, since, in the brains of young or stunted rats, a larger proportion of the bulb lies beneath the cerebrum than in the case of the better developed brains.

2. All layers of the olfactory bulb are about equally concerned in the increase in size or in the arrest of development of the bulb.

3. The small cells of the molecular layer show a larger amount of cytoplasm in large bulbs than in small ones.

4. The number of small cells in the molecular layer, apparently, is not correlated either with age of the rat or size of the bulb. The entire computed number for a small, medium, and large bulb was found to be approximately 1,000,000 cells ± 2 per cent.

5. The mitral cells of small bulbs are smaller, on the average, than those of large bulbs.

6. Within the limits here taken the number of mitral cells is not affected by the age or the size of the bulb.

7. There seems to be some variation between litters in the number of the mitral cells. The average number of mitral cells for 13 bulbs was 76,750, the lowest number being 70,625, and the highest 83,974. The standard deviation σ is 4564 and the probable error of the mean ± 855.

8. When members of the same litter are compared, bulbs stunted by a defective diet or enlarged by exercise show practically the same number of mitral cells as do their controls. The mean difference is -1.8 per cent for the tests.

9. The olfactory bulb size is correlated with cell size and not with cell number.

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ABBREVIATIONS

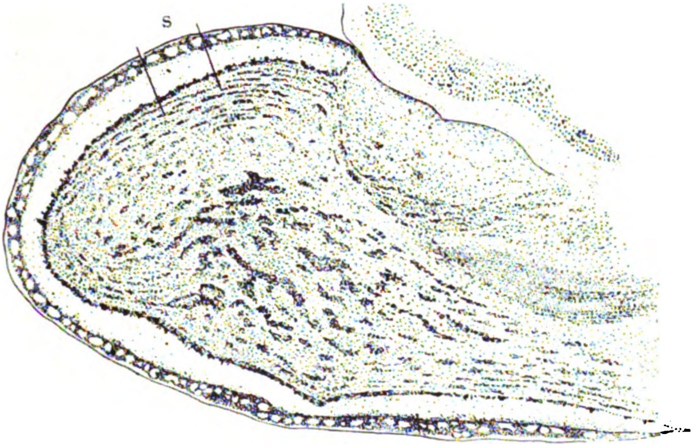
s, areas in figures 1 and 2 enlarged in figures 3 and 4.
fi, outer fiber layer
gl, glomeruli
mo, molecular layer
mi, mitral layer
g, granular layer
f, inner fiber layer
c, cerebrum

PLATE 1

EXPLANATION OF FIGURES

Median longitudinal section through olfactory bulb of F_1, section 3 1/6. F_1, underfed 31 days. Final brain weight, 1.5470 grams, bulb weight, 0.0203 gram. Defective diet. Magnified 24 diameters.

Median longitudinal section through olfactory bulb of F_5, section 5 5/4. F_5, control for F_1. Brain weight, 1.6984 grams, bulb weight, 0.0315 gram. Magnified 24 diameters.



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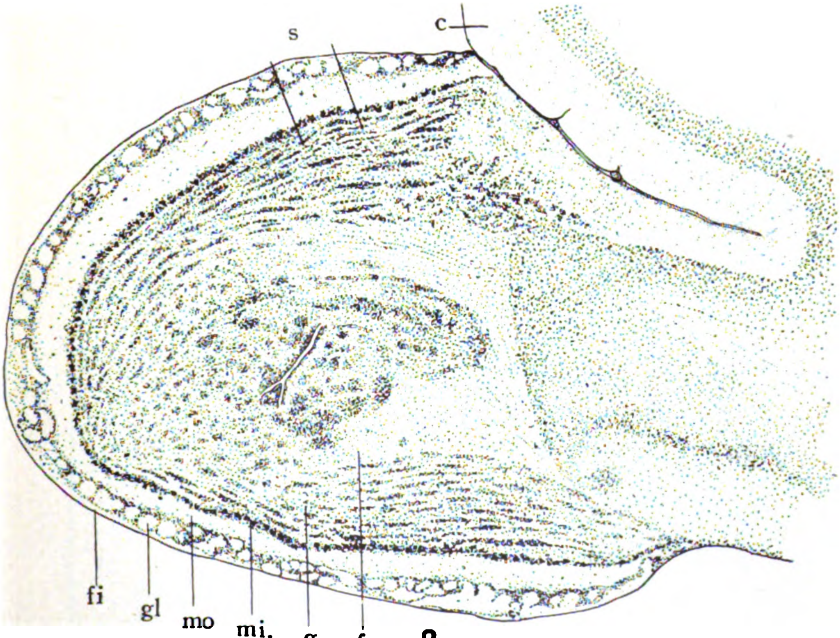
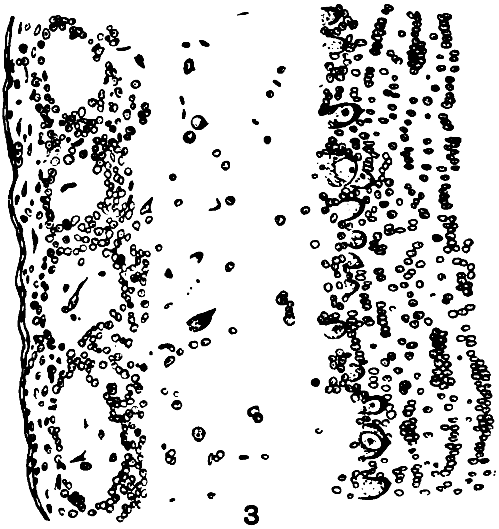


2

PLATE 2

EXPLANATION OF FIGURES

Portion of section of F_1; area S, in figure 1. Magnified 172 diameters.
Portion of section of F_5; area S, in figure 2. Magnified 172 diameters.



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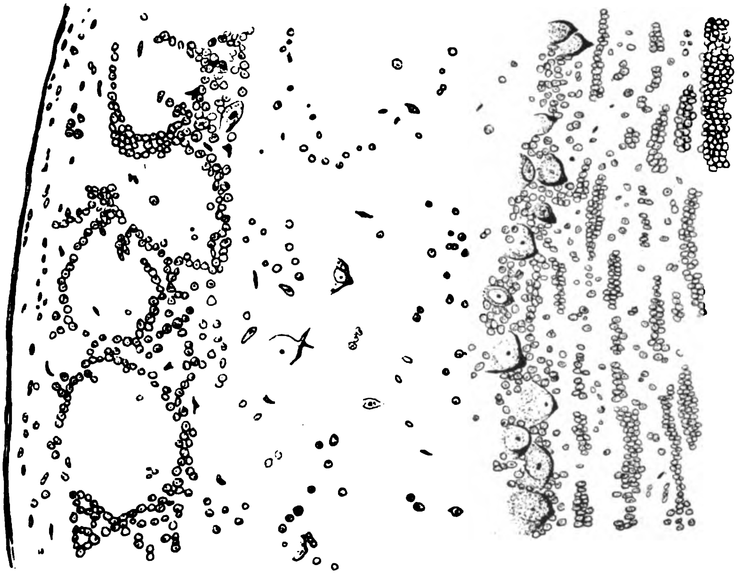


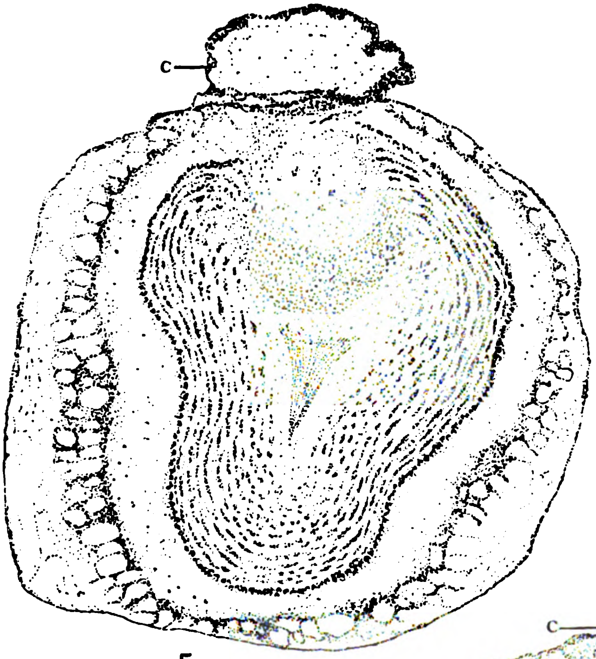
4

PLATE 3

EXPLANATION OF FIGURES

Cross section of Q_5, section 2 3/3, cut at region where bulb joins cerebrum. Q_5, 30-day control rat, killed when weaned. Brain weight, 1.3164 gram; bulb weight, 0.0238 gram. Magnified 30 diameters.

Cross section of M_4, section 4 1/1, cut same region as figure 5 above. M_4, underfed 30 days. Brain weight, 1.4613 grams; bulb weight, 0.0251 gram. Defective diet. Magnified 30 diameters.



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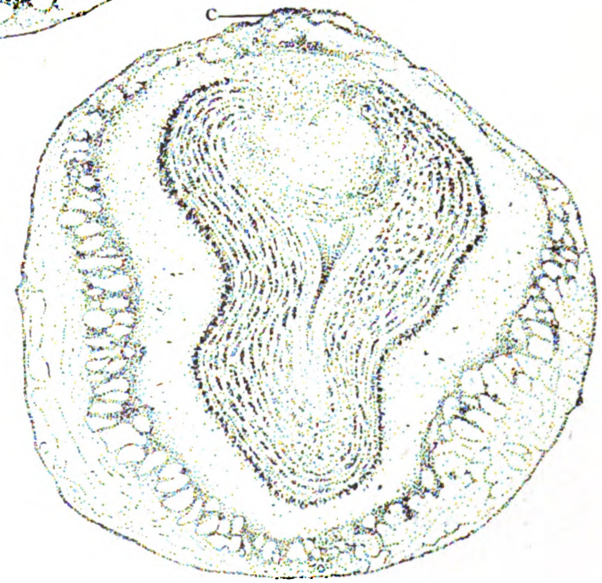
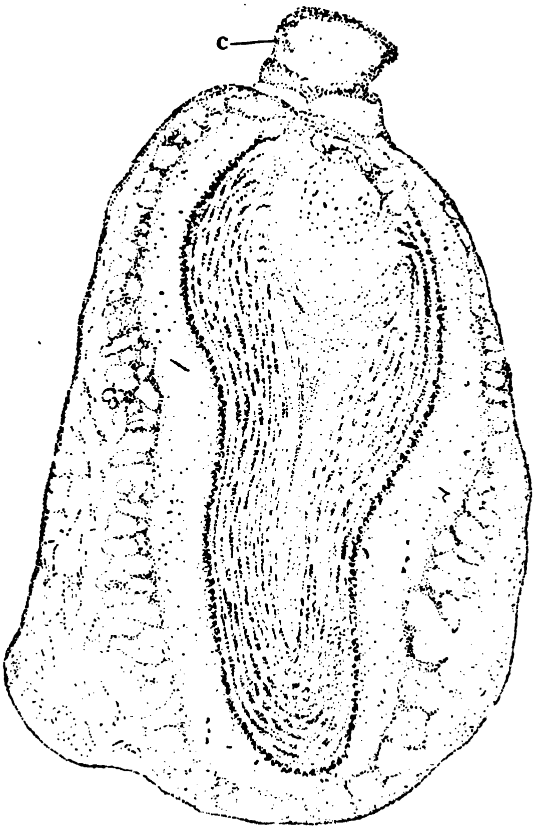


6

PLATE 4

EXPLANATION OF FIGURES

Cross section of M_5, section 3 6/12, cut same as figure 5 above. M_5, control for M_4. Brain weight, 1.7110 grams; bulb weight, 0.0374 gram. Magnified 30 diameters.



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COOPERATIVE TECHNIQUE

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A realization of the very great influence that technique has upon the character of the results obtained in microscopical studies doubtless has come home to every one with extended experience in this kind of work. Time serves only to strengthen the conviction that good methods of preparation are absolutely essential to worthy results. It would be going too far to say that this conviction is universal, otherwise we should have been spared much worthless and troublesome publication, but it can safely be said that there is more general appreciation of the refinements of microscopical methods each year. Up to the present most of our processes are the heritage from the pioneers in this field who gained them through purely empirical means. Comparatively little has been added within recent years. The time would seem to have come to undertake a serious study of the problems in this field with the hope of attaining a fuller knowledge of the processes upon which we are so dependent for all progress in our investigations. It is a most hopeful sign that many biologists are devoting careful attention to this phase of their work and that more precise results are now obtainable than formerly was the case. While much of this advance has resulted from mitochondrial studies, the whole subject of microscopic anatomy has benefited from the greater precision here demonstrated.

But when the individual investigator, thus convinced of the need for advance, faces the prospect of such an extended investigation as would be necessary to developing general principles of operation, applicable to a variety of organisms and tissues, he hesitates to undertake a course which would divert so much of his limited time to methods of preparation. The usual result is that he seeks out the most satisfactory means for handling his own particular materials and contents himself with the best results obtained. Considering the welfare of the whole fraternity of microscopic anatomists this is a wasteful and time consuming method, because it requires, that, in large part, each investigator must work out his own technique and when he has done so his results to a considerable degree benefit only himself.

It would seem that there is a place here for the methods of combination and cooperation which have been so effective in business operations. That this thought is in the minds of not a few biologists is apparent from the inquiries that have come to me regarding the possibility of extending the investigations that are being carried on in the Zoology Department of the University of Pennsylvania to other objects than the ones serving there as objects of study. The increasing frequency of these has led me to consider the advisability of proposing a concerted series of studies upon technical processes, applicable to as wide a range of materials as our investigators have experience with, in the hope of developing more reasonable and more generally applicable preparation methods. In connection with such a series of studies there might be built up at some central place a collection of preparations, each the work of a specialist on some group, organ, tissue or cell, from which would be loaned for study and comparison, such slides as an investigator might need. In addition the results of these technical investigations might be published either separately or collected together and reduced to a more compact form. With the method and its results before him an investigator could determine whether he had attained success in his technique. It is probable that all microscopic anatomists are interested in this phase of their work and disposed to further it, but I do not know how many are convinced that advantage would accrue from a concerted series of studies or, if convinced, are disposed, or able, to cooperate. In the hope of determining whether it is possible to inaugurate correlated studies I am writing this note with the request that any who are interested send me their suggestions upon the subject. If the plan seems feasible I shall be glad to further it in any way possible.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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THE MULTIPLE CHROMOSOMES OF HESPEROTETTIX AND MERMIRIA (ORTHOPTERA)

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EIGHT PLATES

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I. INTRODUCTION

Under the title "The chromosome complex of Orthopteran spermatocytes" ('05) I described certain unusual conditions in these cells, among them the union between the accessory chromosome and particular euchromosomes to form multiple chromosomes. At the time my material was limited and I was able to present only a partial account. Since then I have been accumulating a large series of specimens which have been studied both cytologically and taxonomically. In the course of this study of the more extensive series of preparations a number of facts have been determined which were obscure in the early stages of the investigation. Although it is not yet possible to present a complete study of the two genera, owing to failure to secure cytological material from all the species, there are some facts known which should be presented in order to add what has definitely been determined and to correct errors in the earlier description.

Since the appearance of my former paper ('05) upon multiple chromosomes, a number of observations upon similar structures have been reported by different investigators. Some of these, such as the ones of Voinov ('14), are not clear in their nature, but recent papers by Robertson ('16) and his student, Woolsey, ('16) have made distinct contributions to our knowledge of chromosome relations which will certainly prove valuable. These are strongly confirmatory of the view expressed in my former papers ('05, '08) regarding the persistent organization of chromosomes even in the face of apparent numerical reductions. Very striking is the discovery of a structure, the octad, which I was not then able to find, but whose existence I anticipated so strongly that I gave it a name in advance of its actual observation. The occurrence of such a multiple chromosome I am now abundantly able to confirm, as well as the steps in its formation reported by Woolsey. The underlying principles of chromosome organization, permitting the anticipation of yet unobserved conditions, stands in as striking contrast to the conception of chance association of undifferentiated masses as does the periodic law of chemists to the vagaries of the alchemists.

In this reëxamination, advantage has been taken of the unusual opportunities for a study of the taxonomic characters of the two genera, especially Mermiria, offered by the splendid collections at the Philadelphia Academy of Natural Science under the charge of Messrs. Rehn and Hebard. Much good is sure to come from their active interest in all that concerns the group upon which they are specializing and from their cordial and generous coöperation with other students. By their very careful studies of large numbers of excellently preserved specimens they reach conclusions regarding the relationships of individuals and groups which are soundly based upon well defined external characters and upon personal knowledge of the habits and distribution of the materials. Working quite independently of them I reach conclusions regarding the relationships of individuals and groups from the study of their germ cells and, in most cases, find that there is no difference in our estimate of these relationships. Since the discrimination between nearly related forms, upon cytological characters, sometimes reveals groupings that have been overlooked by earlier taxonomists, the feeling of confidence in the validity of the theories upon which such determinations are made is much strengthened. I feel confident that a full agreement between cytology and taxonomy depends only upon the quality of the criteria of differentiation and upon the accuracy of observation on the part of the followers of these two methods of gaining a knowledge of the organization of biological units of different degrees of complexity and extent.

II. CHROMOSOME CONDITIONS IN THE GENUS HESPEROTETTIX

1. *General observations*

When the observations on Hesperotettix were first announced I had only a few specimens, and, because of unfamiliarity with taxonomic characters, my assistants failed to distinguish the species and confused the sources of the material used for study. It was only when the germ cells were examined that it became apparent that all the specimens regarded as H. viridis did not belong to that species. Later and more careful collections enabled me to determine that H. viridis and H. pratensis were both represented. No difficulty is now experienced in discriminating between these species by germ cell characters. Together with H. speciosus, these represented the full extent of my acquaintance with the genus. Although the chromosome complex was, in its major features, consistently alike in the three species, I published no further observations, because I hoped to obtain preparations from the other North American forms in order to make the presentation complete. After waiting a number of years I am now in a position to give a much fuller account of the chromosomal characters of the genus, although I have not yet seen all the species. Much to my surprise some of the new material shows marked departures from the uniformity prevalent in all my earlier slides. As a result, some of my generalizations are now rendered invalid, but it is hoped that fuller knowledge may make possible the formulation of principles having even wider application.

At present I have preparations from five different species, but in this article I shall discuss fully only the three of which earlier mention was made, for it is only in these that multiples have so far appeared. The number of specimens in each case, except brevipennis, is possibly sufficiently large to be representative.

2. The complex of H. speciosus

The observations recorded for H. speciosus are essentially correct and need only amplification here. It will be recalled that the accessory chromosome is united with one of the tetrads in the first spermatocyte to produce a hexad element. In the spermatogonium the union with one half of the tetrad also exists and is carried over into one of the second spermatocytes. This association was invariable in all the specimens studied, affecting always the same elements. So far as could be observed the unusual relations of the accessory chromosome did not modify its peculiar character and behavior in other respects. The striking appearance of a chromosome, one part of which is condensed and safraninophilous while the other portion is granular and tinged with the violet in Flemming's tricolor, is presented to our view in prophase and telophase of the first spermatocyte. As was pointed out at the time, an apparent reduction in chromosome number occurs without there being any real difference in this respect from other Acrididae. The diploid number of twenty-two becomes the normal twenty-three when it is noted that the accessory chromosome, instead of being free, forms one limb of a V-shaped element, easily distinguished among the twenty-one ordinary rods. That there is here a true preservation of the physical identity of each member of the complex is evident from the continued characteristic behavior of the members which are apparently fused together. The conception of genetic continuity is therefore not merely formal, but expresses the actual morphological conditions of the species. Aside from the presence of the multiple chromosome the germ cells show no marked difference from most other Acrididae.

To complete my former account of the chromosomal characters of this species the following facts may be given: In the spermatogonium appear twenty-one rod-shaped chromosomes which are generally characteristic of the Acrididae. Besides these there is a V-shaped element with arms of unequal length (fig. 9, pl. 3). That this is not a simple euchromosome is indicated by the observation that one member becomes highly vesicular during the prophases. Such a condition is uniformly characteristic of the accessory chromosome under these circumstances and is sufficient to identify this arm of the V as the accessory chromosome. During the synapsis stage there is joined to this heterogeneous pair a third member, the homologue of the euchromosome portion. In the late prophase the accessory chromosome is precocious in condensation and appears as a dense, homogeneous rod, more or less bent, joined to a granular tetrad by endwise union. The contrast is most evident in preparations by the tri-color stain. In the first spermatocyte metaphase this hexad element goes on the spindle with its long axis parallel to that of the spindle and having fibers attached at the ends of the tetrad. This brings one fiber to the point of union between the accessory chromosome and the tetrad. There are visible, at this time, eleven separate chromosomes, of which ten are quite like the ordinary Acrididaean type while the eleventh is the cane-shaped multiple (complex 6, pl. 1).

The anaphase groups resulting from this division each show eleven chromosomes, but in one there is a V which has no like mate in the sister group. This is constituted of the accessory chromosome and one half of the tetrad. In the second spermatocyte into which the V goes, the nature of this is again like that of the spermatogonium—the member which was added during synapsis having been removed in the first spermatocyte mitosis.

The second spermatocytes are accordingly of the usual dimorphic character, one with the accessory chromosome—here permanently joined to a euchromosome—and the other without it. Since the V is made up of a dyad plus the accessory chromosome dyad, it is, in effect, a tetrad, but of unusual type since its parts are not homologous. It divides here just as do tetrads in the first spermatocyte, and the anaphase shows a V going to each pole along with the ten rods. The union of the accessory chromosome with one euchromosome is therefore not lost during all the changes of the maturation period, but persists into the spermatozoon and, by it, may be handed on to the female line of the next generation from which it passes into the male line upon the following fertilization.

3. The complex of H. pratensis

In this species, as in H. speciosus, there is uniformly present a multiple chromosome which differs from the speciosus form only in the proportion of its parts. Without some more definite criterion for the homology of the euchromosomes than we now possess it is not possible to say that it is the same member of the complex with which the accessory chromosome unites in the two species. If size only were used as a basis for judgment, then it would be necessary to say that different tetrads are involved, because the euchromosomes are much larger in speciosus than in pratensis. From the fact that the accessory chromosome itself may vary considerably in different species, it would appear that size is not alone a safe indication of homology. Leaving out of consideration the question of whether it is a particular one of the eleven tetrads with which the accessory is joined, we face the concrete fact that so far as our observations have gone, there is always such a multiple element present in the germ cells of the two species.

When we come to observe the character of the multiple element in the different generations of cells it is found to exhibit throughout essentially the same features as mark its history in speciosus. The only observable difference is in the size of the parts. One individual, however, exhibits a variation in appearance of the multiple, which makes the conditions in Anabrus, already described ('05), directly comparable to those of Hesperotettix (fig. 23, pl. 4). Instead of the accessory being attached to the end of a rod-shaped tetrad it is joined to one extension of a ring. This relation appears in the prophase and is carried over directly to the metaphase, so that multiple chromosomes of very different shape from the ones commonly present are produced. Both forms appear, not only in the same animal, but also in a single cyst. It is probable on this account that the variation does not represent any fundamental difference, but only a divergence in some rate of movement of the parts.

The spermatogonial complex shows practically the same conditions which characterize the corresponding stage in H. speciosus. Minor differences of relative size of the elements are the only ones of note.

Figure 23, plate 4 exhibits the complex in the first spermatocyte. It is at once noticeable that the chromosomes are strongly of the Hippiscus type and in polar view appear as rings or V's lying in the equatorial plate. The only unusual feature is the tetrad, elongated in a plane of the spindle axis, with the accessory chromosome joined to one end at a more or less acute angle. The behavior of the chromosomes during division presents no unusual features and my general description of these types of chromosomes, given at length in a former paper ('14) may be held to apply here. The second spermatocyte mitosis likewise is typical for the euchromosomes, and the multiple chromosome behaves like the same structure in speciosus.

4. The complex of H. brevipennis

There is nothing in the specimens of this species, so far examined, to indicate the existence of any of the unusual conditions of chromosome association which occur in speciosus, pratensis and viridis. As may be seen by an examination of plate 3, figure

8, the complex is strictly of the Hippiscus type. There are neither multiple nor atelomitic[1] 1 chromosomes. It is possible that a more extensive series might show some variation, but this is doubtful. If multiple chromosomes are characteristic of the genus—which might fairly be said from their prevalence in the species so far studied—then brevipennis departs here from the type. Since this paper deals only with the question of multiple chromosomes, no further consideration will be given to species in which they are absent, except to note that the seriation in size is uniform.

5. The complex of H. festivus

This striking and characteristic species, of which numerous specimens have been studied, is like brevipennis and shows no trace of multiple chromosomes. The nature of the complex in the first spermatocyte may be observed in plate 1, complex 2. It seems therefore to have no immediate bearing upon the subject of the present discussion.

6. The complex of H. viridis

This species, like speciosus and pratensis, may possess a multiple chromosome, a hexad, constituted of a tetrad joined to the accessory chromosome dyad. The euchromosome portion is larger than those of the other species. This was the condition present in the first specimens which I studied and which served as the basis for my earlier discussion, although at the time they were not fully identified individuals. Now, with an extensive series secured for me by Miss Carothers, I find that the uniformity of association, so marked a feature of my early slides, has, for certain chromosomes, given way to an order characterized by multiples or non-multiples in equal proportions for the individuals studied. For any individual animal however there is no variation. But this species possesses added interest because of associations between euchromosomes to produce octad multiples in which the accessory chromosome does not occur. This is the first unquestionable instance of any such condition in the Acrididae, but its occurrence in a genus of the Locustidae has recently been reported by Miss Woolsey, working under the direction of Robertson. So clear and unequivocal is this condition in viridis that its interpretation can not be escaped, and that it should have failed to appear in my early material is most surprising. A careful restudy of my preparations makes it evident that no other multiples than those involving the accessory chromosome are to be found there. In some more recently prepared slides of earlier lots I was however somewhat chagrined to find individuals with the accessory chromosome free. These various modifications of the complex are most interesting and suggestive and merit extended consideration.

---

[1] H. viridis. In a former paper I found it convenient to refer to various forms of chromosomes and to note that their forms depend upon the fiber attachment. Following de Sinety, the position of this attachment was designated as terminal, subterminal, or median. In the case of the annular chromosomes terminal attachment apparently results in rings whose plane in metaphase coincides with that of the equatorial plate, while with subterminal, or median, attachment the rings are so placed as to lie parallel with the spindle axis. For convenience these were spoken of as the Hippiscus type and the Stenobothrus type respectively. With the discovery of additional complexity of forms it has been found desirable to have general terms for the two conditions represented by the ring chromosome, and so it has been agreed in this laboratory to speak of chromosomes with terminal fiber attachment as 'telomitic,' while those with non-terminal attachment receive the name of 'atelomitic.' These terms may therefore be used to designate chromosome conditions similar to, but more general than, the ones involved in the two types of ring formation.

The following conditions of the first spermatocyte complex have been encountered so far (plates 1 and 2).

Class (1)–12 separate chromosomes = 11 tetrads + the accessory dyad
Class (2)–11 separate chromosomes = 10 tetrads + one hexad
Class (3)–10 separate chromosomes = 8 tetrads + one octad + one hexad
Class (4)– 9 separate chromosomes = 6 tetrads + two octads + one hexad
Class (5)–10 separate chromosomes = 7 tetrads + two octads + one dyad
Class (5a)–11 separate chromosomes = 7 tetrads + two octads + one dyad and supernumerary
Class (6)–11 separate chromosomes = 9 tetrads + one octad + one dyad

While the number of chromosomes varies from nine to twelve, the number of chromatids, morphologically recognizable, remain in all cases forty-six. The particular form of chromosome which I called an 'octad' in my former study ('05) presents itself very commonly in the new material.

A discussion of these various classes of complexes will serve to bring out most clearly the conditions prevailing in the species. Of these the first shows no unusual conditions whatever, and had specimens of this nature been the only representatives of the genus examined, they would have been regarded as coming from a group entirely typical of the family. There are the usual twelve chromosomes in the form of rings, V's, crosses and rods as found in Hippiscus, all of the telomitic type. The accessory chromosome is free and early passes undivided to one pole of the spindle. Of thirty-eight individuals studied, five had this chromosome constitution.

Seven of the thirty-eight belong to the second class and were the only kind of which I had preparations when my first study was made. Conditions here are as in class 1, with the striking exception that the accessory chromosome is joined permanently to one of the tetrads, forming a hexad element. The structure and behavior of this complex of eleven chromosomes instead of twelve, has been fully described and will not need further consideration here.

Very different is the aspect of the complex in the five individuals in class 3. Instead of twelve separate chromosomes as in class 1, or eleven like class 2 there are but ten. We recognize at once the hexad multiple of the second class and, in addition, eight telomitic tetrads of characteristic forms. The tenth element is much the largest and is otherwise strikingly different from its mates. In shape it is a much elongated ring of the Stenobothrus type, but, like the rings of Chloealtis, usually lacking any lateral extensions. Not infrequently there will be a separation on one side of the ring producing a C-shaped structure. The character of this element will be considered later in connection with other chromosome forms.

Members of class 4, of which I have seven individuals, show still further modifications. The number of chromosomes is reduced to nine, among which are distinguishable six tetrads of usual types, two large Stenobothrus rings and a hexad multiple. The two differential rings are of unequal size. The larger is directly comparable to the similar element of class 3, but the smaller is always recognizable by its minor size and also by its variability of form. In this latter respect it may appear as a fully formed ring, or it may show varying degrees of separation at one of the polar angles, resulting in a large V-shaped structure. Any one individual animal however shows either a ring or a V and this does not suffer variation in any cells.

In class 5, represented by seven individuals, we encounter again the number ten as in class 3, but it is constituted in a different manner. The absence of the hexad multiple is at once apparent, for the accessory chromosome is free and occupies a characteristic polar position. Its sometimes associated tetrad appears among the seven chromosomes of this type and there are in addition, two large rings as in cells of class 4. So far as can be seen, these two elements are as directly comparable with the corresponding two of the preceding class as are similar structures within either of these classes.

To the complexity resulting from combinations of separate elements into multiples, thus reducing the number of independent structures, there is added the opposite condition of a supernumerary chromosome in the animal found in class 5-a. This has a complex similar in its composition to the ones in class five to which is added a small, extra element with the usual characteristics of the supernumerary chromosomes. For the third time there appears the number eleven, but its make-up is different from the complexes of either class 2 or class 6.

The number eleven reappears in cells of the six individuals in class 6, but once again there is lacking the hexad multiple characteristic of classes 2, 3, and 4. Nine ordinary tetrads, a free accessory chromosome and one large Stenobothrus ring make up the complex in this group. In place of the smaller ring, or V, of classes 4 and 5 there are present two rod-shaped chromosomes of corresponding size. The large ring occurs in five of the six individuals and is directly comparable to the element falling in the same place in complexes of classes 3, 4 and 5. The sixth specimen of this group has, in place of the ring, a large V of the same construction as the smaller octad in class 4. This is the only individual in which the larger tetrads were not united at both extremities and, until it appeared, I was inclined to believe that the unipolar union, rather common in the case of tetrads nine and ten, was lacking and that some modification of the association force existed. It is now evident that the conditions are essentially the same in both cases, except that the more frequent occurrence of the large ring and its complete character would suggest a longer history of multiple constitution.

III. CHROMOSOME CONDITIONS IN THE GENUS MERMIRIA

At the time of my first report ('05) upon Mermiria I had studied all the available Acrididaean species and, in every individual, found only one type of chromosome—a telomitic rod in the spermatogonium, which, after synapsis with its mate, appeared in the form of a Hippiscus-type ring or in some modification of the type. Although familiar with the atelomitic form of chromosomes in other material, I had never seen it in the Orthoptera and had no reason to suspect its occasional appearance. Failure to consider the possibility of such chromosome forms was responsible for my misinterpretation of the multiple chromosomes in Mermiria, through which I made the serious error of reporting the segregation of whole tetrads. Although, with increased knowledge of the conditions in the group, it is now possible to determine with certainty the nature of the multiple chromosomes, at the time of my first acquaintance with the phenomenon of chromosome combinations the constancy of chromosome structure and behavior was so marked as to make quite unjustifiable any assumption of variation. Added to this is the fact, which seemed at the time very significant and which even yet is not satisfactorily explainable, that in certain individuals the multiple has a series of definite constrictions corresponding in number and position to what would exist in a decad element. Finally; actual separation in the first spermatocyte metaphase at the level of these constrictions in certain cells persuaded me of their value as an indication of chromosome boundaries. The theoretical difficulties involved in explaining the preservation of the complex on the assumption of tetrad segregation finally convinced me that I was probably mistaken, but it was not until the discovery of the J-shaped chromosome in Trimerotropis by Miss Carothers that any explanation, consistent with the other known conditions in the group, was made possible. Meanwhile I had accumulated an extensive series of specimens and had studied the complex of the female and needed only the conception of the J-shaped tetrad to bring all the observations—with the exception just mentioned—into conformity.

In Mermiria, as in Hesperotettix, the fixity of combinations which seemed to mark the genus has failed to prevail throughout all its species. While such variation as is shown by H. viridis does not obtain in any species of Mermiria studied, multiples are lacking in texana and neomexicana. These results emphasize strongly the necessity of an extensive series of specimens in any investigation—a requisite I have always appreciated and sought to meet. Our recent work in the Orthoptera has shown that numbers of considerable magnitude are required for extensive generalizations.

Mermiria bivittata

It is not my purpse in the present paper to enter into a comparative history of the chromosomes in the various species of Mermiria. I wish merely to consider the multiple chromosome found in bivittata, with its modifications in certain groups of individuals which seem to have specific value. My former report on this species stated that the multiple chromosome consisted of two tetrads joined to the accessory chromosome. This is a mistake the occasion for which is discussed elsewhere. More extended study upon a large series of specimens has shown that in this genus, as in Hesperotettix, there is a hexad multiple instead of a decad. While there is a striking difference in configuration of the element in the two groups, it is due entirely to the form of tetrad involved. In Hesperotettix this is an ordinary extended rod with the accessory chromosome joined at approximately a right angle on one end. Mermiria has, on the contrary, a tetrad already of just this shape, to the straight end of which the accessory chromosome unites. Thus, while the composition is the same, the appearance is very unlike. How this configuration is established, appears very definitely when the chromosome complex of the different cell generations is studied.

Conditions of the spermatogonial metaphase are presented in figures 44, 46, 47, 49, pl. 6. First it may be noted that the number of chromosomes present is twenty-two instead of the usual twenty-three, and that, of these, two differ from the remainder and from the usual spermatogonial rods in being atelomitic V's, with more or less unequal arms. Even a casual study of these differential elements reveals their divergence in both size and structure. One is distinctly larger, and, under favorable conditions, it may clearly be seen that its shorter arm is irregular in outline and more lightly staining. In these respects it conforms to the behavior of the accessory chromosome in many Orthoptera. So characteristic are these appearances that there can be no doubt whatever in the identification of this arm of the V chromosome as the accessory. The V chromosome, then, is a multiple, a tetrad, consisting of the two rod-shaped chromatids of the accessory chromosome joined to the two corresponding rods of a euchromosome, just as in Hesperotettix. At the angle of the V, where the fiber is attached and where the chromosomes join, there is, not infrequently, a clear break in outline. The compound nature of this V is clearly evident from these facts.

Inspection of the smaller V reveals no such indications of heterogeneity, and only in shape does it differ from the remaining elements of the complex. Its position in the metaphase plate is commonly near the multiple V. For convenience of comparison several of these pairs of V's in different stages are shown in figures 47 and 49. The number of chromosomes present in the complex is therefore found to be twenty free telomitic rods of various lengths, two rod chromosomes joined at their inner extremities forming a V and one other V-shaped chromosome—a total of twenty-three, the number characteristic of the family. The internal morphological evidence of the complex in this generation is sufficient proof to establish this conclusion. In addition however we have two confirmatory lines of evidence which are of great value, viz., the subsequent history of these structures in spermatogenesis and the conditions of the female diploid complex. Before following out the later spermatogenetic history I will briefly indicate the conditions in the female cell.

Unfortunately it has not been possible to work out the history of the female germ cells, but the egg follicle cells show beautiful, clear chromosome groups, some of which are represented in figures 43, 45 and 48. It is at once observable that the resemblance to the male diploid group is very marked. Here again there are twenty-two chromosomes, among which are also two V's. A more careful study however reveals a significant sexual difference in the case of the two V's. Instead of being unlike in size they are of practically identical proportions. Owing to their somewhat sinuous course through the cell, with consequent foreshortening, it is difficult, if not impossible, to represent them accurately in drawings. Where they lie more nearly in the same plane, as in figure 43, pl. 6, their equivalence is clearer. Under the microscope there is no difficulty in appreciating the close resemblance existing. On comparing these two V's with the ones of the spermatogonium (figs. 47, 49, pl. 6) it is seen at once that in size and proportion they agree with the larger one of the male cells. In other words these are two multiples, the short arms of which are the accessory chromosomes. The count for the female complex, instead of the apparent twenty-two, is therefore twenty-four, corresponding to the conditions in other species. No differential behavior of the sex chromosomes was observed in these female somatic cells and but little evidence of separation at the point of fusion.

Other species of Mermiria show no multiple chromosomes in the first spermatocyte, and when the spermatogonial complex is observed there are found twenty-three rod shaped chromosomes of the usual type. Absence of a multiple chromosome in the spermatocyte is accompanied by the absence of V-shaped chromosomes in the spermatogonium. Animals of this type are not classified as bivittata, but, quite aside from their exact taxonomic disposition, it is plain that they must be very nearly related to bivittata. In such material, then, the direct relation between V chromosomes of the spermatogonium and multiple chromosomes of hexad nature in the first spermatocyte is strongly suggested. Because of the constancy of chromosome organization, indicated among other ways by the constancy of fiber attachment, we would be justified in saying that the V's of the spermatogonium unite in synapsis to form the first spermatocyte multiple and that this should appear with two nonterminal fiber attachments. Such a condition is realized in the structure of the hexad in the first spermatocyte mataphase.

The conditions of the first spermatocyte complex in metaphase are readily determined, particularly if sections of sufficient thickness are used, and in smears are almost diagrammatic. Here there are clearly eleven chromosomes present, among which is a very large and distinctly different shaped one (figs. 55, 56, 57, pl. 7). This is characteristically in the form of a rod with the two ends of slightly different length bent sharply back in the same plane. One or both of the ends may exceed, or fail to reach, the common angle, producing some variety in form. Greater or less extension, preparatory to division, may result in considerable variation in length, but commensurate and opposite changes in diameter show that the volume remains very constant. Indications of internal composition are afforded by the contour of the element, although, as will be shown later, they are not entirely trustworthy. At each of the bends, where the fibers attach, there is a constriction, and nearer the shorter bent end, at about its length down the shaft, there is a pronounced fissure.

At the time of division, separation occurs at this point, producing two unequal V's in the anaphase. Although the chromosomes are much shorter and thicker than in the spermatogonium, relative proportions are preserved, and if these two parts of the long chromosome (figs. 47*a*, 50*a*, pl. 6) be compared with the two V's of the spermatogonium they will be found to correspond almost exactly. That is to say, two V-shaped chromosomes of certain proportions found in the spermatogonium reappear in the first spermatocyte (united by one limb) and are there separated at this point and segregated into different second spermatocytes. Since one limb of one V is the accessory chromosome, it remains undivided, as usual. Attached to the accessory chromosome is the rod portion of a J-shaped chromosome which has separated from its V-homologue to which it was joined in synapsis. The only differences between Mermiria and most other Orthoptera are (a) the multiple chromosome V and (b) the euchromosome V. That the union of the accessory to a euchromosome is not a fundamental change of the nuclear state is indicated by the fact that certain species of Mermiria lack the association. No change in the distribution of the accessory results from its union, and, as we have seen in Hesperotettix viridis, the combination may be so weak as not to occur in some individuals.

Evidence that the euchromosome V is of like transitional character is not wanting in certain well marked members of this loosely constituted species. In these (figs. 58, 59, pl. 7) the multiple chromosome of the first spermatocyte metaphase is much like that of Hesperotettix except in proportions. There is a pronounced bend at one end at the point of fiber attachment, but the other extremity is almost straight, only a slight subterminal flexure indicating the place of the other fiber insertion. In some instances this point is almost at the end of the chromosome. Individuals with this peculiarity are clearly distinguishable by somatic characters and, I believe, constitute a distinct species. It is possible that, with fuller representation, forms similar in the constitution of their multiple chromosomes to those of Hesperotettix might appear.

This type of hexad caused me much difficulty and led to the conception of a decad chromosome. As will be noticed in figures 58 and 59, pl. 7, there are a number of constrictions along the length of the chromosome quite constant in position, and so placed as to indicate that there are five divisions or parts. It will also be observed that the chromosome may be divided at more than one of these levels. I can not now find any explanation for these separations at various levels on the chromosome, but that they are evidences of unions between various tetrads is not indicated by the full history of the chromosome complex. Again, in the anaphase and telophase of the first spermatocyte, the composite nature of the larger V becomes marked. As may be seen in figure 13 of a former paper ('05), one member of the

V consists of two granular rods of the same character as the remaining chromatin elements, while the other arm is composed of two dense and homogeneous rods of smooth contour. All these parts tend to diverge widely except at the level where the unlike portions join, which is the point of fiber attachment in the preceding cell generations and its site in the one to follow. This difference in constitution is the reverse of the one in the spermatogonium, but corresponds to the relative degree of concentration of the nuclear elements in the first spermatocyte prophase. The interval between the two spermatocyte mitoses is very brief and the chromosomes may be followed through their changes without any loss of identity.

The metaphases of all second spermatocytes show eleven separate chromosomes of which one is a V. On observing these V-chromosomes (figs. 63, 64, pl. 7), however, it is found that they are of two sizes which correspond in proportion to the two V's of the spermatogonium and to the two of the first spermatocyte anaphase. Upon division these are distributed to the spermatids which are accordingly of two classes, equal in number. It is clear from this very evident history of the two V-chromosomes that they go into different spermatozoa and so, upon fertilization, are contributed to different individuals. Of some theoretical interest is the fact that the rod-shaped homologue joined to the accessory chromosome has a criss-cross inheritance while its V-mate is confined to the male line. The presence of the accessory chromosome as a portion of one V means the addition of one chromosome to the count of eleven separate elements, and so conforms to the conditions in other Orthopteran species.

IV. GENERAL CONSIDERATIONS

The very extensive and detailed studies of chromosomes which have been in progress for many years would not be fully justified if the result were merely a record of interesting but non-significant protoplasmic manoeuvers. It is the belief that the substances of the chromosomes are specific materials which are intimately concerned with the development of a multitude of dissimilar cells from a single cell that renders a knowledge of the finest details of their structure and behavior of the utmost importance. That this belief is well founded would seem to be clearly indicated by cumulative evidence from many sources. The fundamental concept involved is the Roux-Weismann hypothesis that the chromatin is the idioplasm, which is differentially organized and linearly arranged. As an indication of this differential organization and linear arrangement, the existence of definite aggregates of the chromatin substance into more or less thread-like chromosomes is regarded as most important.

Details of chromosome structure and behavior are significant, therefore, as indexes of the precision of organization in the material of which they are composed. Since it is the existence and perpetuation of this definite series of differentiated materials that is primarily required by the hypothesis, it is conceivable, and possible, that it may vary in the nature of its aggregates into definite masses (chromosomes) without affecting the manifestation of its various specific effects, except in their combinations. It is not the existence of a certain number of these aggregates that is of first importance, but the presence of the varied materials which enter into their composition. While this is true, it is to be expected that a somewhat exact correspondence should be maintained between the ultimate units of differentiated substances and the units of higher order into which they are assembled. But such a fundamental arrangement may be maintained in the presence of both lower and higher numbers of chromosomes through secondary combinations into units of still higher value in one case, or through duplications of the normal series in whole or in part, in the other. Numerical variation is not of itself *prima facie* evidence of altered organization—it must be shown that something of the complete series is lost, or new and unrepresented materials added, in order to demonstrate the existence of altered organization. The maintenance of the morphologically recognizable units of the original series in the face of changed conditions is indeed added proof of the exactness and stability of structural conditions in the chromatin substance. Evidence of a very important character in support of this position is furnished by the facts disclosed in the study of Hesperotettix and Mermiria. For convenience these facts may be taken up under a number of different headings.

1. *Chromosome numbers in the Acrididae*

It is claimed by those who criticise the so-called chromosome theory of heredity that the maintenance of the specific number of chromosomes is required and that variations in number are direct disproof of the theory. There is an element of truth in this argument which has always been granted by students of cellular phenomena, but stated baldly and without reservation it may lead to entirely erroneous conclusions—indeed has done so in conspicuous instances. The fallacy in the argument lies in the circumstance that a primary organization may be maintained while having superimposed upon it secondary modifications. Instances of this are very common in organic structures. It is no argument against the reality of the pentadactyl type of limb that duplications or combinations or reductions occur in certain groups or individuals. Polydactyl or syndactyl individuals reproducing their modifications of the primitive type are not regarded as illustrations of the absence of a fundamental organization of limb bones. The diplopod condition is rightly considered as secondary to the usual arthropod arrangement—not a disproof of its existence. While it is true that these instances of organization are of a different order from those found in cellular structures they are true examples of meristic variation in individuals in the presence of a persistent type. All that is claimed by those who believe in the hereditary significance of nuclear structures is that the chromosomes are, in themselves, indications of structural organization in the materials of which they are composed. Provided the full complex of elements be preserved, the essentials of idioplasmic control of development exist, even in the presence of combinations or duplications. From this point of view, in the event of apparent variation in chromosome numbers in an individual or group, it is essential to discover whether any of the normal chromatic units are lost or others of different nature added before it can be established that there is not maintained a specific organization.

The belief in such an organization as this has, for brevity, been called the theory of chromosome individuality. This has perversely been much misunderstood or misrepresented. According to some the theory demands an 'independent existence' of the chromosomes, although how this could seriously be maintained in the entire absence of any independently existing chromosome or claim for such, it is difficult to see. In the minds of others the theory demands that the chromosome shall 'make the cell'—whatever that may mean. Of course no such views are entertained by any cytologist and no fair interpretation of the theory of chromosome individuality would give occasion for such statements. Because it is desired to examine the nature of the evidence in favor of this theory, under conditions which might apparently controvert it, a statement of the facts, involved and an outline of the theory may be given to avoid useless discussion. They might take this form in the present state of our knowledge (1) The cell is a complex of organs having various functions; (2) of these the nucleus, is most concerned in reproducing the characters of the cellular organization; (3) of nuclear substances those involved in the structure of the chromatin are most important in carrying out this function; (4) these substances are likewise differentiated and have various rôles; (5) an indication of this differentiation is afforded by the aggregation of these substances into certain definite masses which are characterized by individual peculiarities of size, form and behavior; (6) these aggregates maintain their organization and reproduce themselves in each cell division; (7) their derivatives are characterized by similar attributes of size, form and behavior under given conditions; (8) such complexes therefore occur in all the cells of an individual, both germ and somatic; (9) because members of a species are related by descent their complexes are essentially the same; (10) the fact that a type of organization, which holds invariably for the individual and almost as constantly for the species, prevails with slight variations through genera and even a family is very strong evidence for exactness of organization;

(11) the behavior of the chromosome in maturation and fertilization is strictly in accord with the necessary mechanism for alternative inheritance; (12) the inner constitution of the chromosome is such as to afford an explanation for the linear arrangement of factors and for their variations; (13) in the instance of the most thorough analysis of characters in any one animal, Drosophila, the groups of linked characters and their magnitudes correspond to the number and size of the chromosome; (14) experimental disturbance of chromosome conditions is followed by the expected modifications of characters during development; (15) from the known conditions of nuclear organization in relation to character development predictions with regard to new characters and combinations of characters may be made.

As has been stated before, the number of diploid chromosomes in the various species of Acrididae is usually twenty-three in the male. Exceptions to this have been announced by Granata ('10) for Pamphagus and by various authors for Chorthippus, and by myself for Hesperotettix and Mermiria. Montgomery gave the number twenty for Syrbula acuticornis, but this is unquestionably an error, as Robertson has shown. In no case has a variation been reported for cells of the individual. The gametic complex therefore maintains itself, according to the reports of all investigators. Similar constancy prevails in the species, in almost all instances, except for the wide variation of eighteen to twenty-three here reported for Hesperotettix viridis. In the face of the admitted validity of numerical constancy as one test of the theory of chromosome individuality, how can it be upheld when actual variations of this magnitude exist? This would seem to be as severe a strain as it could be subjected to, and if it can be shown that the conditions in H. viridis are capable of explanation without invalidating the hypothesis, then other cases of apparent exceptions would be less weighty as evidence until their full character became known beyond question.

On first thought the conditions in this group would seem to be a particularly strong argument against the theory. Here is a species, beyond question intimately related to many others of a group in which the number of chromosomes is almost constant, and yet, within the one species, there are wide departures from the normal number. If such changes may occur without producing any effect upon bodily characters that will serve to mark individuals by their variations, then surely it may be argued that there can be no direct relation between chromosomes and somatic structures. This would indeed be a vital objection to the theory if, under its terms, the existence of a fixed group of free chromosomes of unchanging behavior were postulated. Such however are not the conditions of the theory. If it can be shown that the smaller numbers present in some individual are not caused by the loss of any chromatic units, and further that all the conditions in the group are consistent with the maintenance of certain associations between chromosomes and their chance combinations in fertilization, then the conditions of the theory are not violated. In effect this position would extend the theory beyond the observed conditions of the chromosomes to their subdivisions. It would be most exact if it could be based upon constancy of chromomeres, the limit of our observational analysis, as has been done for certain chromosomes in Phrynotettix by Wenrich ('16).

The genus Hesperotettix, so far as our studies have gone, has two species, brevipennis and festivus, in which the normal haploid number in the male is twelve; two species, speciosus and pratensis, in which the number is eleven; and one, viridis, in which both of these numbers are represented and, in addition, nine, ten and thirteen. There can be little doubt that the occurrence of the typical family number in the genus is significant of conformity to type; the presence within one species of this number, and of variations from it, most strongly indicates that, whatever differences there are, they must not be due to any fundamental disturbance of the chromosome organization. This *a priori* argument, while in no sense conclusive, has its value and must be considered. Fortunately however there is strong objective evidence to support it.

First of this evidence is the case where the number is reduced, from twelve to eleven. This I have already considered at length

('02, '05), and the case is supported by observations of Sinety ('01) and others. In this instance an element, so well marked by peculiarities of form and behavior as to be unmistakable in every cell generation, is found to be united to one of the euchromosomes at all times so as permanently to reduce the haploid number to eleven. There is actually one less free and independent chromosome in cells of these animals than in those of other members of the group. This however is a far different thing from saying that there is one less chromosome present. In fact there is no difference at all in the number of chromosomes—the sole variation being the union of the accessory chromosome with one euchromosome. To put the case thus is to make a plain statement of fact and not to create an hypothesis. The apparently missing element is just as clearly present as in any other Orthopteran cells. Irrefutable proof is thus afforded of the process of the fusion of chromosomes without loss of their identity. As a principle of nuclear organization we have therefore to reckon with the ability of chromosomes to unite together while still retaining their morphological integrity.

It is also conceivable that where a tendency of this kind manifests itself between certain chromosomes in a species, it may also obtain between other members of the complex. Such combinations are more difficult to detect on internal structural evidence, because there is no such differential behavior as characterizes the accessory chromosome. Nevertheless there are valid criteria which may be employed and these make certain the existence of such multiple chromosomes. The conditions displayed in H. viridis are peculiarly fortunate and may now be considered in detail. From the study of thirty-eight individuals of H. viridis it is apparent that (*a*) the number of chromosomes in the first spermatocyte may vary from nine to thirteen and that (*b*) the same number may be present in two or three individuals and yet in each case be differently constituted. The necessity for a careful and detailed study of the complex before passing judgment upon the significance of numerical variation, is certainly apparent from these circumstances. In view of the fact that most explanations of changes in the number of chromosomes have concerned the smaller members of the series, it is of much interest to find that in Hesperotettix this portion is relatively stable while the larger elements may be variously combined together. But however much difference there may be in numbers there is apparent no loss of any morphological structures in any of the classes. A comparison of homologous series of chromosomes, based on size, may be made on inspection of plate 2 where I have attempted to arrange the chromosomes of different classes in order so that homologous chromosomes are in vertical rows. In the case of multiple euchromosomes they are placed in an intermediate position between the columns where their members would properly come. The accessory chromosome, when in a multiple, is drawn as it is attached to its tetrad.

Even a casual inspection of this plate will show that, aside from the accessory chromosome, the seven smaller chromosomes, (nos. 1 to 8) constitute a graded series in which variation is not great. The eighth chromosome, however, may or may not have the accessory chromosome attached to form a multiple. In classes 4 and 5 there are above this chromosome only two other separate ones, but these are of a character to attract attention at once. Unlike most of the remaining elements of the complex, they lie extended, parallel with the spindle axis and are in the form of open rings, each half of which is greater than the whole of chromosome eight. There are a number of considerations which make it practically certain that these are multiples composed respectively of chromosomes 9 and 10, 11 and 12.

First, it is to be noted that if these are counted as octad multiples, the number of chromosomes becomes exactly twelve. In weighing the value of this piece of evidence it must be remembered that this number is characteristic of almost the entire family to which Hesperotettix belongs. This fact makes it certain that in this respect we deal with such a fundamental feature of organization as to require us to consider any variation, not as *prima facie* evidence of a lack of precision in organization, but rather as a modification of the form of it.[2] In the

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[2] One of the most unjustifiable and unscientific attitudes imaginable is that which would regard the effort to interpret the form of organization in its various aspects only as an attempt to force a real divergence into a seeming agreement. Such a style of argument has been resorted to by a number of those whose belief

case before us the individual animals in class 1 have twelve chromosomes, those in class 4 have nine while the ones in class 5 have ten. The question we have to decide is, whether these conditions represent absolute differences in organization, or whether, within the nine and ten groups, the twelve elements of class 1 are morphologically present. On inspecting a complex of class 4 we find that the accessory chromosome is missing from its usual position in the series, but upon looking further it is discovered, with all its usual characteristics of structure and behavior, attached to one of the tetrads. As a structural element of the cell it is demonstrably present and must be counted, although it is not a free chromosome. In a complex which is of almost identical composition otherwise, (class 5) the accessory chromosome is a free element and would of course be counted. In both cases the entire history of the accessory chromosome in all cell generations is typical, whether free or attached. The remaining two chromosomes of classes 4 and 5 are strikingly different from all the others, and upon careful inspection are found to have morphological subdivisions, which if counted in the usual way, restore the apparently missing two. In other words, if the number of chromatids is counted the total is forty-six, just as it is in class 1. So far as the number of elements is concerned, therefore, we are dealing with the same series in each case. It is obvious that if the differences in number were the result of an unordered variation there would be every reason to expect numbers in excess of twelve, together with modifications of all the elements and not of precise changes in particular ones.

In a similar way, when the numbers in classes 2, 3 and 6 are considered, the same evidences of ordered change are seen. Only eleven separate chromosomes are found in class 2 but the largest of these shows very clearly the accessory chromosome as a con-

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is that the chromosomes are unstable and indefinite structures of the cell. Back of such a position is the implication that evidence must be taken at its face value without interpretation. This I consider entirely wrong. The essence of observational work is interpretation, and in microscopical investigations it reaches its fullest development. The value of evidence submitted by a microscopist is in direct proportion to the quality of his interpretative ability.

stituent part and thus demonstrates the completeness of the series. In class 3 there are ten separate chromosomes, of which one is a multiple involving the accessory chromosome as in class 2, besides which there is a very large octad similar to the one in classes 4, 5, and 6. Class 6 differs from 3 only in respect to the non-union of the accessory chromosome with a tetrad. The one individual of class 5 a, of which I have only a smear preparation, presents the second instance of a supernumerary chromosome which has appeared in my material up to the present. Aside from this, the complex is like that of class 5. From all these considerations I think it may be said that the numerical variations within these specimens of H. viridis, instead of being an indication of lack of stability in organization of the chromosomes, are, in fact, very strong proof to the contrary. There is nothing whatever to indicate that the integrity of any element is lost, or, aside from the case of the supernumerary, that any additional members are added to the complex. Variation is not a question of loss or gain, but of relations.

2. Chromosome numbers in general

Variations in chromosome numbers, within the species and larger groups certainly exist, as is clearly manifest in the work of numerous investigators. The constancy of numbers is more definitely established in some groups of animals than in others. Thus the Acrididae have a common number for many of the genera, but in the Hemiptera, as Wilson and Montgomery have indicated, the families are much less uniform numerically. In using the conditions of the family which I have most studied as an indication of chromatin organization, I have recognized that the nature of this organization might be different in other groups, and I have not attempted to apply generally the immediate conclusions to which I inclined. Not only is this true but the specific statement to that effect appears in an early paper of mine ('08 a) in these words:

> I am quite prepared to admit also that in one species even, there may be a variation in the integration of the chromatin material, resulting in some numerical variation of the chromosomes, without losing

my belief in the necessity for this definiteness in the grasshoppers. We do not yet know how much difference there may be in the organization of the various chromosomes of a complex nor how variable in importance they may be.

A more careful reading of my papers would have saved some of my critics much futile argument.

While I have thus avoided any consideration of the general topic of chromosome variations in other papers, I should like now to examine some of the data relating to this subject. It is important that this be done, because most of the attacks upon the theory of chromosome individuality have proceeded from investigators who either found, or think they found, variations in chromosome numbers within individuals or species. Such attacks have run the gamut, from assertions that chromosomes are merely physical aggregates without morphological value, down to questions regarding the definition of the term chromosome. In undertaking such a general discussion I shall base my opinion largely upon material with which I have personal familiarity. Much harm has come from attempts to homologize results from widely different materials by persons who have no first hand acquaintance with the conditions discussed. With our present knowledge of cellular phenomena as slight as it is, such lengthy critiques, involving the interpretation of other investigators' interpretations, can accomplish little good and may greatly retard progress by fixing attention upon relatively unimportant details. A striking instance of this has been the controversy regarding pre- or post-reduction, extended long past the time when it was definitely known that the question of the segregation division is one of the individual chromosome and not of a whole mitosis. The matter of chromosome organization is, however, fundamental to all our present conceptions of the cell in relation to the larger problems of biology, and the evidence is cumulative that the essential assumptions of our hypotheses are justified. On the other hand it seems clear that the nature of chromosome integration varies with different groups and that, for this reason, we must be cautious in carrying over the conclusions reached from a study of one population of organisms to another. It is in full realization of this need for care in generalization that I wish to examine the conclusions of others regarding chromosome numbers in the light of my own and my students' studies.

In making such an examination I can profitably take up only the most general questions, because, in matters of detail, it becomes a case of interpretation of phenomena, and many times one is not justified in criticising the work of others without himself knowing the objective conditions. For this reason I should like to consider the work of Della Valle on the subject of chromosome numbers. I do not choose this author as representative, because of any inherent strength apparent in his papers, nor am I impressed by their number and size. On the contrary they appear to me weak, because of the small amount of observational basis and the large development of theory. For instance in the paper of 1909 of the hundred and seventy-seven pages, only forty pages are included under the heading "Data of observation" and, of these, twenty are concerned with matters of technique. Throughout all of his publications, indeed, there is apparent a painful lack of judgment regarding the distinction between fact and theory, and were he alone in his position he could fairly be dismissed with little attention.[3]

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[3] 'Objective analyses,' accomplished by the translation of morphological facts into terms of physical chemistry, carry their own indictment of lacking experience and judgment, and could safely be left without comment. Such an attitude toward chromosome organization is, however, but an extreme case of the position assumed by a number of biologists who would seek to discredit the hard won facts of cytology by an appeal to conditions or forces beyond observational control or by an arrogation of the whole problem to their own chosen field. It would seem that, in almost every case where such attitudes are assumed, there is some animus or prejudice in the mind of the writer which betrays itself in his injudicious or intemperate language. All those who fail to agree with his particular extreme views are depicted as banded together for the establishment of dogma and the suppression of truth. With much vigorous language and the plentiful use of exclamation marks all such are consigned to scientific oblivion with their obsolete methods and narrow views.

This is very unfortunate. It is rarely the case that scientific investigators are not honestly in search of the truth with regard to the subject of their enquiry, and to accuse them of conspiring for its suppression is most absurd. It is true that new views sometimes prevail but slowly, but it does not hasten their

The work of Della Valle is open to criticism from two sides. It may in the first place, well be questioned whether the knowledge of colloid chemistry, and of fluid crystals in particular, is sufficiently established to justify the extension of its principles into the operation of cellular phenomena. Certainly one is inclined to doubt the basis on which such application is made when the complicated phenomena of chromosome division are declared to be 'absolutely identical' with the cleavage of fluid crystals, or when the involved changes of the chromatin in the telophase are described as 'identical' with the solution phenomena of a gelatin cylinder in warm water. It is of course not to be doubted that in the activities of the chromosomes, and all parts of the cell indeed, chemical and physical laws are operative. Such a belief is however far removed from the one which conceives some particular manifestation of chemical or physical energy as 'identical' with the behavior of the chromosomes in mitosis. Much might properly be said with regard to this phase of Della Valle's work, but I desire rather to consider the nature of what he advances as direct evidence against the theory of chromosome individuality. Since this involves a thoroughgoing denial of all the facts and theories relating to the subject, such a discussion will touch upon most of the objections that have been raised by other critics and will avoid the necessity for repeating arguments involving only minor differences of material or opinion. A summary of his position with regard to matters relating to chromosome individuality follows:

He asserts that the number of chromosomes in a cell is due to the constancy in the amount of the chromatin and the median size of the single chromatin aggregates. This number suffers variation according to the law of fluctuating variations and is

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acceptance to attack the motives of those who hold other opinions. A method does not commend itself as an instrument of value merely because it is termed an 'objective analysis' when it is obviously the application of a series of analogies. Neither does an observed fact cease to be such on being termed a 'hypothesis' or even a 'subhypothesis.' It is in fact not infrequently true that much of good in the work of certain biologists issues under a severe handicap because of the inherent evidence of poor judgment in their estimation of values in the work of others.

subject to conditions within the system of which the chromosomes are a part. The chromosomes are temporary and variable organizations of the chromatin, which form in the prophase and dissolve in the telophase. Variations in size of chromosomes are within the limits of observational error and are of the order of size variations in the droplets of an emulsion. Correspondence in size between chromosomes and nucleus finds its explanation in the physical condition of adsorption. The fact that chromosomes shorten proportionally to their original length shows they are homogeneous and indicates the identity of all the chromosomes in a mitosis. The previous history of the chromosomes in a line of cells can have no effect upon later generations because there is no continuity. Definite or specific organization is lacking and there is no perpetuation of a series through reproduction of individual chromosomes. All explanations of variations in number (called 'sub hypotheses') are declared to be untenable upon 'accurate examination' and certain determination of numbers in sections is stated to be impossible. As is customary with critics of this type, Della Valle, after decrying the spirit and purpose of those who do not agree with him, proceeds to attack their methods and declares that only his own technique yields infallible results. Sections can not give accurate enumerations of chromosomes, and only counts made upon stretched membranes are of value. Having thus put out of count the bulk of the work already done, he next makes a comparatively limited number of observations upon one type of material and arrives at the ultimate general conclusion that chromosomes are fluid crystals. With this solution of the problem accomplished, all minor questions are easily settled, because upon this major premise that chromosomes are fluid crystals and subject to all the laws known to pertain to such physical aggregates, certain variations of number, size, form and behavior must exist. I should like now to consider some of these assertions in the light of work known to me personally, first pointing out that all instances of order and system under varying conditions are direct evidence against his position.

The number of chromosomes within cells of an organism, he states, is variable, according to the law of fluctuating variations, because the number in any cell is due to the constancy in the amount of chromatin and the median size of the chromatin aggregates. Opposed to this is all the evidence, already given, regarding the high degree of regularity in the Acrididae where so many genera are alike in number, although the amount of chromatin varies widely. In this large group, the family, variation is infrequent, but in one species, Hesperotettix viridis, variation is common. Certainly under the terms of Della Valle's argument the greater variation should occur where there are the greater differences in amount of chromatin. But if there is any truth in the assumption, some variation would be expected in the cells of the individual and this does not occur in these Orthoptera. As reported for Culex by Whiting ('17) in the germ cells the diploid number is constantly six, Hance ('17) finds the same conditions generally true of the somatic cells, but Holt ('17), working upon the same material, discovers a range of variation extending from six to seventy-two in certain intestinal cells. It is very clear that the amount of chromatin is here not constant, while the median size of the chromosome remains practically unchanged. There is no correspondence, either, between the size of the nucleus, or of the cell, and the number of chromosomes. No evidences whatever of any balanced physical system, such as Della Valle advocates, appears in these cells of Culex.

Hertwig's outworn nuclear-plasma relation theory receives just as little support. In H. viridis the amount of chromatin is much the same in all the cells of a given generation and yet the number of chromosomes in the haploid condition ranges from nine to thirteen. Along with this fixity in the amount of the chromatin and variation in chromosome numbers, goes a constancy of size series up to certain chromosomes, beyond which there is a sudden change. The phenomena relate to no means or averages, but concern definite morphological entities. In place of fluctuating variations there is definite and determinable order. What happens is explainable, not upon circumstances of chance relations between masses of substance in different 'phases,' but through a knowledge of the history of certain individually recognizable chromosomes traced through a large group of animals. Worthy of note is also the fact that in such cases as H. viridis the range of variation is strictly defined and falls within the limits set by the organization established in the family, except in the occasional instance of supernumerary chromosomes—two instances in the thirty-eight individuals studied.[4]

It is only upon the basis of entire identity of all chromosomes in a complex and of their temporary character that the Italian author has any argument at all. Building upon this he seizes upon every reported instance of difference of chromosome numbers as a support for his thesis. Every descriptive fact is labeled a 'sub hypothesis,' and so many of these are secured in this way as to convince him that the main hypothesis of chromosome individuality has no standing. Curiously enough, he conceives a great importance for the fact that one explanation for all the reported cases of numerical variation is not sufficient. On the contrary, he says, there are so many ways of accounting for such variation that there can be no constancy and no individuality. Perhaps in no other way is the quality of his argument better indicated than here. The fact that an error in observation has been made, and even admitted, is only an indication of variation; when it is shown that a reported difference in number is due to the inclusion of more than one species in a study this is a subhypothesis weakening the main one; the production, by hybridization, of chromosome numbers different from those of the parents is accounted as an example of variation, as is the case of asymmetrical mitoses in neoplasms. The clear cut description by Wilson of the failure of certain chromosomes to enter into synapsis is not an explanatory fact but another 'subhypothesis.' Similarly, early separation of conjugants, delayed divisions of certain elements, the presence of supernumeraries are merely so many condemnatory 'subhypothesis.' The exact determination of the union of the characteristic and well marked accessory chromosome, with a tetrad to form a multiple is not a fact at all, not even a hypothesis, but only a 'subhypothesis.'

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[4] If a chromosome is but a chromosome without character or distinction, there is in this case a violation of the rule stated. But if the nature of the element be significant, the rule holds, for the entire history of the supernumerary chromosome demonstrates that it is of a different order from the euchromosomes. It may in many ways depart from the history of typical chromosomes and finally end in complete elimination. Arguments, such as Della Valle's, require that such a structure be considered identical with all other bodies called chromosomes, without in any way regarding its individual history of ultimate extinction.

From this it is clear that Della Valle, and others like him, have set up in their minds the conception of an absolutely fixed and invariable number of chromosomes of constant and unchanging form as the hypothesis of chromosome individuality. This is a man of straw, fathered by no biologist of standing or character. His destruction in no way affects the existence of the real image of a constancy in chromosome organization consistent with our other knowledge of the living substance of which these structures are a part. Such misinterpretation merely condemns the judgment of the one who has no better discernment of the real problem. It seems incredible that a biologist should be capable of twisting the truth in such a way as to turn an investigator's meaning entirely around so as to make his results support a converse. Only when his basic conception of chromosome constitution is fully appreciated does an explanation of Della Valle's attitude appear. This is expressed by him in several places in some such terms as these: The chromosomes are temporary and variable aggregates forming in the prophase and disappearing in the telophase ('09). Between two mitoses the chromosomes are so completely lost as individuals that finally no trace of them can be found ('13). It is easy to see that, with such a view of chromosome organization, no constancy is possible and the quickest way to dispose of variable numbers is to consider them the result of chance.

But such a disposition of the case neglects certain objective facts which can not be disposed of by consignment to the class of 'subhypotheses.' It has long been known that the accessory chromosome, and other heterochromosomes, preserve their morphological identity from one generation of cells to the other. All through the spermatogonial divisions each chromosome lies in its own vesicle and can be clearly recognized. Recently Wenrich ('16) has carefully traced certain differentiated chromosomes in Phrynotettix through much of the history of the maturing germ cells, without loss of their physical identity. Instead of appearing as "temporary and variable structures" they are just the opposite, being persistent and exact in organization to the highest degree. Not only are the chromosomes constant in number, but also, and more fundamentally, the chromomeres. Such conditions are probably unknown to Della Valle and are certainly unappreciated or he could not make such sweeping assertions regarding chromosome instability.

Perhaps one of the most striking and extensive instances of variation in chromosome numbers yet reported is the one described by Miss Holt ('17) for the intestinal cells of Culex. Here, in one individual, there may be a range from six to seventy-two. Without extending the analysis of the case beyond the simple determination of the numerical conditions, one would be justified in believing that no significance attaches to the mere number of the nuclear bodies. This would follow on the major premise that indefinite and fluctuating variations in numbers are direct evidence of lack of definiteness in organization. This might presumably be admitted for the somatic cells without entirely invalidating the theory of individuality so far as it applies to the germ cells, for it is possible that, among other evidences of differentiation in cells, correlative changes in the nature and integration of the chromatin substance might occur. Especially might this be the case in pupal Culex intestinal cells, because these are on their way to disintegration in preparation for the new epithelial lining of the enteron. Meanwhile the germ cells of the species preserve an unvarying constancy in number. But, as Miss Holt shows, the matter is not so simple as it appears. True, there is variation, but it is not indefinite and unordered. There are not always six chromosomes present, but, if more occur, the numbers are multiples of three and repeat in corresponding series the sizes of the primitive complex. Not only this, but the grouping is maintained and the derivatives of each of the original series remains associated with its fellows and, with them, forms a common vesicle. So far as prophase and telophase conditions are involved it would not be observed that anything unusual in numerical relations obtains. Even in the matter of this multiplication the chromosomes exhibit individual differences, and one may have advanced in the process beyond the others and in this way produce a total number not strictly a multiple of three. But no matter how many or how few there may be, each one of the individual groups repeats the characters of the original member. How far removed from mere chance crystallization out of an indifferent matrix is the ordered and definite reproduction of distinct morphological entities which we see here. In the very face of what seems at first glance to be the grossest of variability there appears only another marked instance of exact organization. It is of interest to observe also that division of the chromosome may occur independently of mitosis, which suggests, in connection with the observation that the actual splitting of the chromosome is a prophase change, that mitosis may be more concerned with cell division than with chromosome separation.

3. *Chromosome sizes*

If the total amount of chromatin is constant for each cell and if the number of chromosomes is unchanged, it might reasonably be expected that the same series of sizes would always appear. These conditions are certainly realized in the individual, where the closest correspondence exists between the series and between individually recognizable elements. That like correspondence exists between individuals seems probable when the seriation in the complex is noted. An exact determination of chromosome sizes is however a very difficult undertaking, owing to the variety of forms they assume. One is forced to estimate relative volumes, and an exact determination between two nearly equal sizes of different form can not be made with certainty. Fortunately the difficulty of comparing sizes is less in the case of the larger elements, owing to their volume and more uniform shape, and we may be sure of the series in this part of the complex. Since it is between these chromosomes that combinations occur, the members may easily be identified. The value of the evidence with regard to the constancy of chromosome organization as shown by size relation is best appreciated by a comparison of the different classes shown in plate 2.

In the one containing twelve chromosomes (1) there is a gradual increase in size from the smallest to the largest without any marked breaks. Class 2 shows the same condition, with the exception of the multiple chromosome, but if the presence of the accessory chromosome there be disregarded, the disproportion vanishes. Class 3 however presents a different aspect. There is the same gradation up to the largest element, which, in turn, is more than twice the size of the next one of the series. Similar conditions prevail in each of the other classes, with additional distinctness in classes 4 and 5 where the element succeeding number 8 is like the largest in class 3. In each of these cases there is an abrupt and disproportional increase in size, if these structures are regarded as simple units, which entirely disappears if their multiple constitution is recognized. Such jumps of size in the complex are lacking in any species of this family where the twelve chromosomes are present, and it is not reasonable to consider that they represent any great change in the organization of the chromosome such as would be involved if these huge chromosomes were in fact of the same value structurally as the lower ones of the series. However much difficulty there may be, for example, in locating chromosome 4 or 5 in the series, there can be no mistake made in judging the relative size of the largest element in classes 3, 4, 5 and 6. It is also easy to see that either half of this ring exceeds the dimensions of the next chromosome in size in somewhat the same degree as chromosome 11 does chromosome 10 of class 6. Similarly the members of the smaller ring in class 5 are related to chromosome 8 of class 6. The relations in size of the upper members of the series are demonstrated most conclusively by a comparison of them in classes 4, 5 and 6.

While these unequivocal conditions certainly obtain and justify the effort to homologise the chromosomes on the basis of size, it must be admitted that there appears to be some fluctuation in volume, both absolute and relative, between the same members of the series in different individuals. How much this may signify it is difficult to say. There are so many ways in which the size of a chromosome may be affected in the processes of microscopical technique that a just estimate of the significance of size variation is hard to reach. Thus it is known, for instance, that very marked differences in size may result from the method by which the animal is killed. If this be true, there are doubtless other ways in which these delicate structures may be similarly affected. Since however the animals themselves may vary greatly in dimensions without in any other way modifying their character, the same may be true of their chromosomes.

A very careful study needs to be made of chromosome dimensions, based upon material prepared with the utmost refinement of technique and studied with most careful mensuration. Under ordinary conditions there are many circumstances which would serve to obscure differences between nearly related members of a series and make their identification uncertain. While this is true, most of our observations would indicate that agencies affecting the size of chromosomes operate more or less uniformly on the entire complex, so that a proportionate change is found in each element. With the subject as difficult as it is there is no wonder that views as widely divergent as those of Meek and Della Valle exist. The former author thought at one time that he had detected a uniformity so great that only two diameters appeared in all the Metazoa, but a more extended experience did not confirm this opinion. It is true that there is much uniformity in the diameter of the chromosomes of a complex, and Meek's careful measurements disclosed this; but, on the other hand, there are extensive movements in the chromatin substances and the diameter varies inversely with the length. There is always the possibility of such movements and the extent of them at any given time is variable, so that we may expect to find differences in dimension although the volume remains constant.

Meek has done good service in pointing out the facts, but it is unfortunate that he should push his generalizations so far upon a limited experience. Very different are the methods and conclusions of Della Valle.

Variations in size, according to Della Valle, fall within the limits of observational error and are of the order of size variations of droplets in an emulsion. Correspondence in size between chromosome and the nucleus finds its explanation in the physical condition of adsorption. As in the case of numbers, the appeal is from an explanation based on order of determinable character to one in which only the chance circumstances of physical relations obtain. Opposed to this conception stand all the careful investigations in which definite size relations are shown to be preserved, not only in a single series but in a duplicate one. Within this occur occasional inequalities, which, in turn, are of definite character, pertaining to certain chromosomes and so recurring as to be accounted for most readily through genetic continuity and chance distribution and recombination. Upon this point again the circumstances in H. viridis offer important evidence. The smaller members of the complex form an ascending series which, in class 1, is continued without breaks through the larger members. But in the remaining classes there are sharp breaks in seriation of exactly the same character in all the cells of the individual, entirely beyond the range of observational error and past the possibility of chance variation. The conditions are altogether of the character of order and system common to living structures and foreign to purely physical phenomena. The material upon which the Italian author worked is not nearly so favorable for size determinations as are many other kinds, because of the great length and irregular contour of the elements, but even here much more accurate results could have been obtained by the exercise of such care as was employed by Meek. From some studies being pursued in this laboratory by Mr. Parmenter I feel certain that the Amphibia are no less exact in their size relations than are other groups of animals.

4. Chromosome forms

In a previous paper ('14) I have considered at length the subject of chromosome forms in the Orthoptera and do not here desire to go into the subject beyond what is necessary to an understanding of the multiples. A glance at plate 2 will show how strongly these elements differ from the tetrads in metaphase. The typical Orthopteran chromosome is telomitic and, in polar views of the spermatogonium and second spermatocyte metaphase, it appears as an approximately straight rod which, in the anaphase, becomes divided into daughter rods of similar nature. In the first spermatocyte these rods, joined in pairs to form tetrads, show a great variety of shapes in metaphase, but in the anaphase again become reduced to the form of two rods joined at one end to form a simple V, the enclosed space of which lies between two chromatids of the former tetrad. Upon division of the hexad in the first spermatocyte of Hesperotettix this condition is again realized for the rod-portion, but opposite this in the other anaphase group stands a V, the arms of which are not simple rods but double ones, and the enclosed space of this lies, not between chromatids, but between whole chromosomes. The nature of this double-V I have considered fully in former papers. Its peculiarity of form is due to the fact that fiber attachment is non-terminal—a condition resulting from the union of non-homologous chromosomes and not from a real shift of the fiber. All this is very clear in Hesperotettix, and the character of the double V is beyond question and easy of determination. From this one would be inclined to generalize and to say that where fiber attachment is shifted from the terminal position, producing double V's in the first spermatocyte anaphase, it is due to the union of non-homologous chromosomes. Of course to make this a valid argument there would have to be a corresponding reduction in the group number of free elements. This condition is fully met in Hesperotettix and is confirmed by the differential morphological characters of the elements involved. In the absence of such criteria based upon structure, the sole test regarding the character of the double V's would be that of numerical conformity.

Upon turning to Mermiria bivittata we encounter these conditions: a spermatogonial complex of twenty-two free elements among which are two atelomitic V's. If it were assumed that the latter are always multiples, then the male diploid complex would be twenty-four. There are two arguments against this conclusion however. First there is the circumstance that the genus—a well marked one—has species with the normal twenty-three free chromosomes. The value of such evidence has already been considered. A second piece of evidence is the actual morphological structure of the V's. One of them shows by all its history that it is directly comparable to the similar structure in Hesperotettix—it is a multiple composed of the accessory chromosome and a rod-shaped euchromosome. The other V is found in the first spermatocyte attached to this multiple element. Unless this combination be of greater valence than a hexad, the second V can be only a dyad. Strong evidence that this V is indeed only a simple dyad appears from a study of certain individuals which I have called the 'green' bivittata. In these specimens the multiple chromosome is almost the shape of the one found in Hesperotettix, the fiber attaching near the free end instead of toward the middle (figs. 58, 59, 60, pl. 7). The valence of the part is very clearly indicated in this way. Owing to mistaken enumeration in the spermatogonium, because of insufficient material, I regarded the multiple as a decad instead of a hexad in my earlier work. In this opinion I believed myself confirmed by the persistent type of chromosomes in the family. It was only when new material revealed to Miss Carothers that, in a full complex of twenty-three chromosomes, there might be numerous double V's, and that one such an element might be united to a rod, producing a J-shaped tetrad, that I found it possible to account for the form of the Mermiria multiple. With plenty of favorable material I was also able to determine accurately the diploid number of chromosomes in the male to be twenty-two. Having all these facts it was not difficult to solve the problem.

It is apparent, therefore, that form alone is not sufficient to establish the nature of a chromosome. The double V may or may not be a multiple. In Mermiria, in the same chromosome of the first spermatocyte, we have two such V's, one of which is multiple, the other not. In Trimerotropis double V's are numerous although the complex is not reduced in number. But the form of the chromosome, taken in connection with the number in the complex, the relative sizes of the elements, their structure and behavior, is important. With these limitations on the value of chromosome form in mind, I wish to consider the evidence they afford regarding the nature of the Stenobothrus type of rings and V's in H. viridis.

In such a study the occurrence of the incomplete ring is very helpful because it constitutes an intermediate form between the two rod condition and the ring. An examination of these elements in classes 4, 5 and 6 (plate 2) will demonstrate these relations. In the last class, chromosomes 9 and 10 are rods arranged on the spindle parallel with its axis, and there divide at the middle constrictions. Similar structural conditions prevail in class 4, with the exception that, in place of chromosomes 9 and 10 as free elements, their position in the size series is occupied by a single chromosome in the form of a V placed in the same relation to the spindle. Upon division, the elements of this unusual chromosome separate at their middle constrictions just as did free chromosomes 9 and 10, and in one of the anaphase groups exactly the same conditions obtain as in cells of class 6 (fig. 28, pl. 5). The other group has, in place of two rods, a double V corresponding in the number and size of its subdivisions to the sum of those in the two rods from which it separated. All the circumstances of the case indicate that the only unusual condition here is the union of chromosomes 9 and 10 at one end, producing, in the diploid condition, a V, whose arms are unequal in size and otherwise non-homologous. The origin of such a combination has not yet been determined, but it is not a temporary condition during the maturation period, for it is found in all generations of the cells and is undoubtedly present in the somatic cells. Since it is present in half of the sperm it may as readily perpetuate itself as the two rod condition. If like relations exist during the maturation of the egg, then a means for producing all the chromosomal arrangements found in H. viridis is at hand. Thus a two rod spermatozoon and a two rod egg would produce a complex having chromosomes 9 and 10 free as in classes 1 and 6. A double-V spermatozoon and a two rod egg would bring about the condition shown in class 4 where these chromosomes are joined at one end, while such a spermatozoon, united with an egg of similar composition, would result in the complete ring of class 5. It seems evident therefore that the forms of chromosomes found in the male germ cells may be accounted for on the basis of persistent fusions, segregations and recombinations.

Similarly, if chromosomes 11 and 12 are compared, we find the two rod, incomplete ring, and ring, and the argument for their relation is the same as for the preceding case. There are however two notable differences between the series. Of the six classes, only two show multiples of chromosomes 9 and 10, while four classes have combinations between 11 and 12. Again, the incomplete ring is as frequent as the complete in classes 4 and 5, while only one individual out of twenty-six belonging to classes 3 to 6 failed to have a union of the two tetrads at both ends. Thus we learn that the tendency of the large elements to unite is more pronounced and complete. In this connection it may be noted that in class 2 the largest element, although failing to form a union with a euchromosome, is united to the accessory chromosome. Only four individuals of the thirty-seven show this element not joined to another chromosome. Of similar import is the combination between the accessory with the largest free euchromosome in class 4. It is possible that the largest free tetrad is involved in class 3, although I judged it to be next to the largest when the drawing was made. Another thing to be noted is that only tetrads of nearly equal size unite together. The accessory chromosome is never joined to a chromosome of lesser size.

The form of the multiples, as represented on the tabular series of plate 2, is the one best adapted to serve as a means of comparison between them and the free chromosomes of other individuals. This relatively simple configuration is, however, not the only one assumed, and some of the figures would be difficult of conception in the absence of acquaintance with the possible range of chromosome movements. For a number of reasons these variable forms are of importance and require consideration. It is to be noted that this extended ring may be the final shape assumed by other metaphase forms just previous to the anaphase, or it may represent the status attained by chromosomal movements antecedent to the metaphase. Probably all that is involved throughout is a difference in relative movements of the chromatids. An examination of figures 14*b*, 16*c*, plate 3 will reveal a chromosome, one part of which is extended over the spindle as are the members of the flattened ring already described, while, embraced between its ends, lies another ring within the plane of the equatorial plate. By comparing this chromosome with others shown in photomicrographs Q–V, plate 8 it is seen that this second ring may be progressively reduced until it corresponds in form and position to the extended element to whose ends it is attached—the sole remnant of the equatorial ring being an enlargement at the middle of the curved rod. It is apparent that the enclosed spaces in these two rings, lying at right angles to each other, can not be the same morphologically, unless the chromatids in synapsis have shifted. One must represent an interchromosomal space, the other the longitudinal split of the chromosomes. In this respect the element is similar to the double ring figures in certain Orthopteran tetrads.

Another form of not infrequent occurrence is similar in outline to the hexad of Mermiria and consists of a curved rod applied conformably to the spindle, with the fibers attached at the ends. From these ends also there spring at various angles more or less straight chromatin rods equal in length, approximately, to half the curved rod to which they join. Superficially such an element is almost the counterpart of the Mermiria hexad, but is nothing more than the large extended ring whose shorter half has divided at the equatorial constriction in advance of the longer (fig. 24). A more puzzling case is that of the curved rod extending from one pole of the spindle to the other and almost equalling the combined length of the two halves of the ring or V (fig. 19, pl.

4 and photomicrographs P., Y., pl. 8). Such a structure would result if the V were unfolded on the spindle with fibers attaching at each end, but in this case the parts on either side of the equatorial constriction would be unequal and division at this place would mean separation of whole tetrads. Because of the relatively slight difference in size of the joined tetrads, I have not yet been able to satisfy myself of the exact relation of parts in this elongated chromosome, but I suspect that separation of chromatids has occurred in a plane at right angles to that which obtains in the V. A full study of the prophase history should clear up this matter.

Emphasis needs to be placed upon the statement already made that the mere form of the chromosome is not conclusive evidence, either of its valence or its method of division. Valence can be determined only by a study of the element through the various generations of cells and in numerous individuals of the species. Planes of division are certainly identified only by some morphological character which marks one of the synapsed elements. Instances of the exactness in configuration of chromosomes of tetrad, hexad and octad valence have already been given, but it would seem desirable to consider the ring chromosome a little more at length. In former papers I have called attention to the importance of such elements as indicators of the planes of division, and in one ('14) I pointed out the distinction between the Hippiscus and Stenobothrus types and indicated how failure to recognize such distinctions has led to much confusion. At the same time it was recognized that, in the presence of parasynapsis, the opening in the ring might represent either the space between homologous chromosomes or their longitudinal division. Since there was, so far, nothing to suggest two types of ring formation, it was considered that only one exists and the available evidence would indicate that the annular space lies between homologous chromosomes. In the presence of one type of ring there must necessarily be two forms of first spermatocyte division, for the Hippiscus ring divides along the length of the ring while the Stenobothrus type separates transversely. Owing to the fact that, up to that time, all rings of the Stenobothrus type has been found in complexes of less than the full twenty-three numbers, some uncertainty existed regarding the connection between these two circumstances. That this connection was not necessarily causal was indicated by the case of the unidentified 'Stenobothrus-like' species with a complex of twenty-one and numerous rings of this species-type. But not until the work of Carothers on Trimerotropsis was it certain that there is no necessary relation between the Stenobothrus ring and lesser chromosome numbers. It is now clear that this form of chromosome is not in itself evidence of multiple constitution above the tetrad. Structurally all that is involved, apparently, is an atelomitic fiber attachment.

But if it is not true that such rings and the double-V's which unite to form them, and which reappear upon their division, are criteria for octads, it is very suggestive of such composition to find them so frequently in cases of reduced numbers. Where it can be shown that progressive steps in such unions occur within a species, as in H. viridis, and that morphologically recognizable divisions of these elements which, if counted as units, exactly restore the reduced number to that characteristic of the species and family, then the form of the chromosome is of value in determining composition. Especially is this true if by such means always the exact number characteristic of the group is restored, and if, in groups of different chromosome numbers, the distinctive numerical relations are preserved. All of these desiderata are realized in Hesperotettix of the twenty-three chromosome family and in Jamaicana of the thirty-three and thirty-five group as reported by Woolsey. The parallel between the two cases seems to be very exact, with the exception of a more marked tendency toward combinations in Hesperotettix.

The circumstances appear unequivocal in the cases of Hesperotettix and Jamaicana where multiples may or may not exist within one species and where intermediate steps indicate the method of combination. The question which next presents itself is with regard to the application of these criteria to cases, such as Chorthippus, where there appears to be a permanent and fixed reduction of number, not only for the species, but also for the genus and related genera. In a recent paper Robertson ('16) has considered this matter with great care and has advanced strong arguments in support of the position that the family number may be maintained in the face of apparent reduction. His argument may be thus summarized. (1) The number of chromosomes for the family is twenty-three in the male; (2) the form of the chromosomes, when uncombined, is that of a rod with terminal fiber attachment; (3) size relations are constant and valid indications of homologies; (4) in the presence of less than the typical number of chromosomes certain ones of the complex are V's with non-terminal fiber attachment; (5) if the limbs of the V's are counted as units, the number twenty-three is restored; (6) the behavior of the limbs of the V's in maturation is parallel to that of corresponding free elements in other species of the family; (7) the morphological composition of the V is indicated by a non-chromatic bridge at the angle where the fiber attaches.

I believe that in the species studied by Robertson the criteria are largely applicable and the conclusions essentially justified. The evidence is, to a considerable extent parallel to that of H. viridis, with the exception of intermediate steps in linkage.

There is clearly a permanent reduction in the number of free chromosomes—it then becomes a question as to whether there is a definite loss of morphological entities in the chromosome complex, or whether all the twenty-three elements are potentially present, even though in combinations. It must be recognized, I think, that, however fundamentally alike are the cases of Hesperotettix and Chorthippus, they stand on a somewhat different basis morphologically. In the former, independence of certain chromosomes is strongly evidenced by their free condition in some individuals and their partial union in others; in the latter the number of free elements is constant, not only for this genus but for others nearly related, and evidence of combinations must be more indirect. Considering the criteria which I have employed in Hesperotettix and Mermiria I would regard the following as probably applying in the case of Chorthippus: (1) The number of chromosomes, so strongly indicated in the family, is made exactly normal by counting the limbs of V's as units, (2) the graded size series, sharply broken if the V's are considered as simple units, is restored if they are compound elements, (3) the form of the univalent chromosomes is so generally a rod in the family that the occurrence of V's, in connection with the circumstances in (1) and (2), is strongly suggestive of multiple constitution. To these should be added the conformatory evidence of cases like H. viridis which establish on direct observation the principle of chromosome combinations.

That these circumstances justify the generalizations which Robertson draws regarding the multiple nature of V-chromosomes in general seems much less certain. Because of the great importance which attaches to the exact determination of chromosomal conditions in the germ cells it is essential that our cytological evidence be most carefully considered. I should like therefore briefly to indicate where it appears that limits should be placed upon the extension of the principles indicated by conditions in Hesperotettix, Mermiria, Anabrus and Jamaicana. Since Robertson has made the widest application of these by his conception of the multiple constitution of V-shaped chromosomes, an examination of his presentation will illustrate the nature of the difficulties attending such generalizations, into some of which I was myself led. It is fortunate in this connection that there is no question regarding the accuracy of the observations. His work is very carefully done and the drawings are clear and definite.

Agreement has already been indicated with the first two premises of his argument, i e , that the number twenty-three is typical for the family, and that the form of the univalent chromosome is a rod with terminal fiber attachment. Like most other biological generalizations, however, these are not without exceptions in the Acrididae. Pamphagus, with telomitic elements, has only nineteen chromosomes and Trimerotropis with numerous and variable V's has a full complex of twenty-three. My unidentified Acridian reported in a former paper ('14) (which may be a species of Circotettix) has nine atelomitic chromosomes in twenty-one chromosomes, while Circotettix, as reported by Miss Carothers, has similar conditions of less than the typical family number and more than enough V's to compensate. Despite these exceptions the value of the evidence for constancy of number and form is strong because of the few departures from type.

With regard to size as a test for homology there is much less certainty. No chromosome is more definitely to be determined than the accessory chromosome and this falls in no constant size position, either for the family, genus or species. Robertson reports it varying in size from number 1 to number 5 in the Tettiginae and from number 8 to number 10 in the subfamily Oedipodinae. It seems to vary from number 3 to number 5 in the genus Hesperotettix and from number 4 to number 5 in the species viridis. Variation in size of the individual chromosomes, recognizable by other morphological characters, has been reported by Wenrich ('16) after a most careful study of Phrynotettix. In this latter case variation appears to be the result of actual loss of certain parts of the chromosome, but in general it is due to the extent of condensation and possibly to the amount of more fluid substance held within the chromosome. All our observations indicate the existence of a fairly definite series of sizes throughout the group, and the extreme members of the complex can not be confused, but it is clear that no certain identification of neighboring elements can be made by size alone. For this reason, even if the achromatic bridges did mark the limits of joined chromosomes, it would not be certain that specific numbers united as is indicated by Robertson. Here it may be noted that in H. viridis chromosomes of nearly equal size, and the largest in the series, are joined together. Practically the same conditions are reported by Woolsey in different species of Jamaicana. Judging from her figures I should be inclined to say that it is not improbable that neighboring chromosomes, and those of the largest size, are united here. Measurement of the chromosomes as drawn is not a very accurate way of determining their size, since the amount of foreshortening can not be known, but it is so frequently the case that the element marked '14' in her figures of the multiple, is clearly larger than the free number '15,' that the possibility of confusion exists.

The significance of the form of V-chromosomes, as an indication of multiple constitution, is bound up intimately with the question of numbers. Only when the limbs of the V, counted as units, with the rod chromosomes, total the number characteristic of the family can there be such evidence of multiple constitution, according to the terms of Robertson's argument. If it appears that such elements exist under other circumstances, then it is necessary to assume that there may be more than one type of V. This Robertson does and uses as a criterion for the V, indicative of multiple composition, the presence of a distinct achromatic bridge at the apex of an acute angled structure. The final test of the validity of his generalization becomes therefore a structural one. There is no doubt of the accuracy of his determination of such a condition and he has with great care traced it through all the stages of maturation. It is unfortunately true, however, as Carothers and Wenrich have shown, that equally clear non-staining bridges occur in atelomitic chromosomes of Trimerotropis where the full complex of twenty-three is present. Conversely, in cases where undoubted combinations exist, as in H. viridis, no distinct achromatic bridges at the point of fiber attachment appear. This seems to be true of Jamaicana also, judging by the figures of Woolsey and of Robertson himself. It seems certain therefore that the V-form of chromosomes, is, in itself, no indication of a multiple condition. If this be true for the Acrididae it is quite useless to consider its value in other groups where the chromosome constitution is less well known.

The form of certain first spermatocyte chromosomes and the behavior of their parts in this mitosis is regarded by Robertson as further confirmation of his belief in their multiple character. He draws a close parallel between them and free elements of other species through homologies of size and form. Since the limits of these supposed elements are fixed by the achromatic bridges at the point of fiber attachment, their validity as tests for chromosome boundaries is questioned for the same reasons as were given above. I should however like to speak more at length regarding this form of chromosome—the double rings.

These are represented very exactly in Robertson's ('16) figures 174, 179, 180 and 182, and the achromatic break is considered the point of union between tetrads. I believe that the weight of evidence supports this interpretation: that these are indeed potentially multiple chromosomes, but at the same time I feel that there is no justification for the belief that this form of chromosome is the result of the union of two tetrads of such shapes that this configuration results. My evidence for this is that just such shapes occur in full complexes of twenty-three chromosomes, of both telomitic and atelomitic type. They are found in many species and have been figured by Sutton, Granata, and myself. As I have pointed out, the two rings lie in planes perpendicular to each other and their origin is easily conceived if the chromosome is constituted of four parallel rods. A similar conception appears in the interpretations of the other two authors cited. The same difference between double rings of the telomitic and atelomitic types holds as in the case of simpler annular chromosomes of the two types, for the fiber attachment is persistent and determines the position of the chromosome on the spindle. Therefore such structures are very much alike in Trimerotropis and Stenobothrus and are so placed on the first spermatocyte spindle that the ring to which the fiber attaches lies parallel with its axis, while in forms with telomitic chromosomes only, such as Hippiscus, Tropidolophus, Brachystola and others, the ring to whose 'lugs' the fibers attach lies in the equatorial plate. Such differences are however entirely independent of simple or multiple composition of the diploid chromosomes.

Finally I consider them valueless as criteria of multiple constitution, because in Chloealtis, a genus nearly related to Chorthippus, the same numerical and size relations obtain, and there are no double rings to suggest preservation of chromosome forms such as are found in more widely removed genera. So far as the form of the chromosomes is concerned, the conditions in the Tryxaline, Chloealtis, are much more nearly like those in H. viridis, of the Acridiinae, than they are in Chorthippus, a very nearly related genus.

At this point it may not be out of place to speak of the condition of cross segmentation as an index of chromosome boundaries, since this is a matter that has led many, including myself, into mistakes. These non-chromatic segments are doubtless definite indications of structure in the chromosome and probably are always of the nature of the spaces lying between the chromomeres, as in the 'selected' chromosomes described by Wenrich ('16). Such regions are more pronounced in appearance and more persistently free of chromatin where the fiber attaches than elsewhere in the chromosomes, but probably do not differ otherwise. Even this distinction may be lacking, as in Mermiria (figs. 58, 59, pl. 7) where the chromosomes seem not infrequently to separate at different levels. It was this strong cross segmentation at definite levels, with subsequent division, that led me to believe in the decad constitution of the multiple in Mermiria.

Another thing which has contributed to Robertson's misinterpretation of the V-chromosome is his belief that only in their presence do pachytene loops appear in the nucleus. No one feature is more characteristic of the prophase condition in all Acrididae (from which the Tettiginae should be removed, as I suggested in 1908 and as Robertson believes) than the bending of the chromosome so that both ends lie at one side of the nucleus. Even the univalent accessory chromosome takes this position, as was shown in an earlier paper ('02, fig. 12). Wenrich finds that the looped condition is not confined to the large elements, but is characteristic of the small chromosomes of which he made careful, detailed study. It is of interest to note that, in this respect, the members of the octad in H. viridis behave as though they were independent, each forming its own loop. This is a very strong indication, indeed, of their multiple nature. The conditions at this time in the Tettiginae are unique so far as my experience goes Here, as Robertson states, the chromosomes are extended in a more or less straight line, and since they are much longer than the nuclear diameter, the whole nucleus is drawn out, producing very irregular shaped structures. It was this circumstance, of which he had detailed knowledge, that led Robertson to believe that loops are unusual.

The form of the chromosome is conceived by Della Valle to be uniformly that of a rod with rounded ends. Such is frequently the shape of metaphase chromosomes, particularly in the diploid condition, but no one who has studied the complicated changes through which the members of the haploid series pass at the synaptic period, could entertain the simple conception of a homogeneous rod spontaneously dividing along its length, advocated by this author. The most remarkable and striking phenomena of biology are entirely ignored by this explanation. Precision of organization, shown by Wenrich to extend to the limit of our observational powers, is entirely disregarded by the gross conception of a formed and homogeneous rod cleaving like an unorganized colloid mass. The extensive and significant changes of the prophase, when the division actually occurs, receive no recognition in this crude explanation of chromosome division. The form of the chromosome is a much more involved question than would appear from the statement of the case by Della Valle.

5. Chromosome behavior

Of very great importance for the theory of chromosome individuality is the behavior of the elements united into multiples. If the chromosomes are merely colloid masses—products of the cell—it would not be expected that they should exhibit any differential characters. The very fact that such differences of behavior exist is a disproof of all theories which postulate complete loss of identity and reformation of chromosomes at each mitosis. Unless there is continuity of substance there can not be continuity of organization, because we know of no organization independent of material basis. Besides, such persistence of structure and substance is observable. As indicating the high degree of this specificity of chromosome characters, the multiples are of much value, especially when they involve the accessory chromosome. If all the elements of a mitosis are 'identical' and 'homogeneous,' as Della Valle asserts, there would not be the marked difference in behavior of the euchromosome and the accessory chromosome which we always witness in the Orthoptera. Most especially would this be true where the two types constitute a single mitotic element. What a contrast the actual facts regarding exactness and differential character of the chromosomes present to the crude and vague generalizations of those who conceive the cell to be only a microscopic laboratory for the play of the chemical forces involved in colloidal crystallizations. It is however fortunate that there are minds not content with a half way denial of the facts relating to nuclear constitution and the substitution of indefinite generalization about the activity of the cell as a whole or of specific enzymes, etc., but which push the denial of precision of organization to an unconscious *reductio ad absurdum* in some field of chemistry. For through this service we are brought to see the contrast between clearly defined facts, gained through researches of the most exacting and painstaking character and the uncertain and contradictory theories springing from impatient and prejudiced minds.

The facts regarding the differential behavior of the chromosomes, together with those relating to numbers, sizes and forms, are, many of them, now commonplaces of observation and are regularly determined by beginning students in microscopic anatomy. Others, such as those exhibited by the euchromosome multiples described in this paper, are no less striking and significant. They all speak clearly and unequivocally in favor of an order, in terms of morphology, and against one of a more remote and molecular character. But no single feature of chromosome behavior possesses the significance and fundamental importance which attaches to the unquestioned reproduction of each individual chromosome during mitosis. Why, unless there be something in the organization to be preserved, should the elements so carefully reproduce themselves down to the most minute structural peculiarities? It is inconceivable that the complexity and exactness of this process should exist unless there is some correspondingly important function to be carried out.

If the chromosomes are homogeneous and identical, as Della Valle asserts, their substance could most easily be divided while they were in 'solution.' The complication of the mitotic process is all out of proportion to the simple end to be served under such an assumption.

6. Chromosome distribution

In most mitoses it would seem that every step of the process is directed toward securing the exact distribution of the chromatin elements. The outstanding exception to this rule has been the independent movement of the accessory chromosome in one of the maturation mitoses, where it goes into one cell entire without division. This is clearly a differential mitosis, featured by an asymmetrical distribution of the chromosomes. One of the elements possesses a distinctive character not shared by the others—it has an individual and more or less independent movement which takes place at only one time in all the history of the organism. Up to this one point it is distributed in mitosis like the other chromosomes, but just here it betrays the inherent difference of its nature. So much of the chromatin substance acting as a unit in mitosis, possesses distinctive characters. Are these the consequence of this separate unity, or is there some specific nature of the material? The history of the hexad multiple chromosome answers this question definitely in favor of the latter alternative, for, although joined to another element, the same characteristic features of behavior and distribution mark the accessory chromosome as when it is free. It is incomprehensible that there should be this definiteness of action on the basis of mere chance—such a conclusion is foreign to all our experience with living structures. Taken in connection with the parallelism between the development of sex and sex linked characters, and its distribution during maturation, all the history of the accessory chromosome speaks for specific organization and self perpetuation. The fact that it violates the practically universal rule of a complete division of all the members of the complex in each mitosis in order to accomplish its necessary distribution and self perpetuation is an unanswerable argument for its specific individuality. That this distribution is properly maintained, although the element is no longer free in the multiples, is only another confirmation of the conclusions otherwise determined.

Another evidence of specific organization through acts of distribution comes from the studies, upon unequal and heteromorphic chromosomes by Miss Carothers ('17). Organization, expressed in size or form, is perpetuated by a distribution and a conceivable recombination according to chance movements. There is no indication that any indefinite variation exists. While it is true that no explanation of the origin of the heteromorphic condition is strongly suggested by the facts so far observed, it may be noted that Wenrich's work affords objective evidence regarding inequalities in size. Always a means for the maintenance of given conditions appears in the mechanism of mitotic distribution.

The same precision in distribution which marks the accessory chromosome is true also of the elements which unite to form the euchromosome multiples. No difference exists between the distribution of these members of the complex, whether free or combined. It might be thought that the intimate and apparently permanent association of two chromosomes, resulting in the formation of a new mitotic unit of different value, would materially change the activities of the components. So far as I can see this is not the case, and even the details of movements in the prophase are preserved in the face of the multiple condition.

The problem of accounting for the occurrence of a given variable series of heteromorphic chromosome combinations in Trimerotropis has been considered by Miss Carothers and she properly arrives at the conclusion that segregation and chance recombination of components offer a competent explanation of the observed phenomena. At the same time there is not excluded the possibility of a readjustment of relations between the chromosome complex and the new cytosome at the time of fertilization, which, within a limited range of possibilities, would result in a similar variable series. The conditions within the species of H. viridis would not be fully met by the explanation through segregation and recombination, without assuming a heterogeneous character of the material or a lack of survival of certain classes. While both of these possibilities exist they are remote, and the phenomena, taken in their entirety, are much more suggestive of a reconstitution of relations in the new individual, probably at its origin on fertilization. This is a problem which concerns the most fundamental activities of the chromatin substances, and about which we lack information on all the stages of fertilization and maturation of the egg. Breeding experiments should serve to decide between the possibilities suggested, and these will be carried out, but the nature of the processes will not be clear until the complete cycle of the chromosome complex has been studied. This fact has been realized since the beginning of the work on the Orthoptera, but the technical difficulties involved are great and so far have not been overcome.

While, therefore, it is not possible to draw any definite conclusions from our present knowledge, it may be desirable to direct attention to the conditions which confront us. Reasons are given elsewhere for considering the individuality of the chromosomes as well established by the observed uniformity of number, size, form, behavior, etc., but in the case of the multiple chromosomes we face a series of variations. As has been pointed out, variability is a universal property of living matter and is a subject for investigation, not an evidence of lost individuality. Taking into consideration, first, the accessory chromosome, whose individuality is marked so definitely as to be unquestionable, we find, that, within the species H. viridis, it may possibly vary slightly in size although this may be only apparent. From this we would draw the conclusion that, for the species, size is a fairly safe criterion of homology, although not absolutely exact. Using size to identify homologues, then, we discover that the accessory chromosome is joined to the largest chromosome in class 2 (plate II), to the third or fourth from the largest in class 3, and to the fifth from the largest in class 4. With persistent union, segregation and chance combination it is apparent that there could be no union between a spermatozoon of class 2 and an egg of classes 3 or 4, for instance, without synapsis of non-homologuos chromosomes. Still using size as a test for homology, we find no such indication of non-homologous chromosome unions in this species.

This difficulty entirely disappears, however, if we assume that the formation of multiples takes place at fertilization. Such a view is in strict accord with our knowledge of the importance of this step in the production of differences between individuals and of constancy of character exhibited by each of these variants throughout its subsequent history. Also there is offered a ready explanation of the formation of multiples, which would, at the same time, explain the variety of elements sometimes involved, as in this case; for if there be a period at which chromosomes are brought into intimate relations in a linear series, multiples would be merely persistent cases of such associations. The contrary might also be true, the multiples showing a tendency to overcome forces keeping the chromosomes apart, but, in view of the variety of elements involved in H. viridis, this would seem less reasonable than the first explanation. There are also other considerations, into which I have not the time to enter here, which incline me to believe that multiples indicate the operation of a common principle of chromosome association. The alternative for this explanation of the conditions in H. viridis, by reason of associations established at the time of the union of the spermatozoon and egg complexes, is the assumption that, at some time, each of the classes represented in plate 2 became established in some way and has since maintained itself by the segregation of its elements and their chance recombinations, with the elimination of all the unrepresented conditions. By this we are still left without an explanation of the first instance of each class and must confront a selective mortality of considerable magnitude, for which there is no evidence.

Such an explanation, as that to which I incline, for the conditions of the multiple chromosome in H. viridis does not make impossible another for the case of Trimerotropis, and it may well be that in the latter instance, which concerns questions of fiber insertion or other variation between homologous chromosomes, the simple method of segregation and chance recombination obtains, while in H. viridis, where non-homologous chromosomes are involved, another mechanism is operative. Genetical experiments will shed some light on these difficulties and it is hoped that these may be completed before long.

7. *Chromosome individuality*

As has been stated elsewhere in this paper, the circumstances relating to the multiple chromosomes are strong proofs of the theory of chromosome individuality, as indeed are all facts indicating order and definiteness in form and behavior. So far as I can see there is no half way ground between the assumption that the chromosomes are definite, self-perpetuating organic structures and the other which presents them as mere incidental products of cellular action. According to one view individual chromosomes are descendants of like elements and possess certain qualities and behavior because of their material descent, the visible mechanism for which is the process of mitosis: according to the other any similarities that may exist in the complexes are the result of chance aggregations of non-specific materials. It is a choice between organization and non-organization in the last analysis, at least in terms of cellular structures. To attempt the substitution of a conception of molecular organization, which is beyond the experience of the biologist and which exceeds the present powers of the chemist to analyse, is to cast aside all hope of solving the problem of cellular action, because it is necessary to understand, not only the physical and chemical phenomena involved, but also their different forms in the various parts of the cell. It is readily admitted that these are not the same in the nucleus and in the cytosome, but some hesitate to recognize differences between parts of the nucleus, and more are disinclined to grant specific activities to the chromosomes.

Since it is not possible to observe directly the action of the chromosome we are obliged to make use of indirect evidence, seeking parallels between elements of structure and action in the chromosomes, and the mass effect of cellular action as exhibted in the so-called body characters. Such a method is justified by all our other experience in tracing relations between structure and function in organisms, and while it apparently resolves the individual into parts of greater or less independence, has given us our best conceptions of it as a whole. Homologies have been much more significant of relationship than analogies when concerned with organic parts, and I do not doubt that conclusions drawn from structural relations will greatly exceed in value the remote and equivocal analogies between nuclear structure and colloidal phenomena. On the basis of our experience we anticipate a commensurate relation between structure and function, but this does not mean a fixed and exclusive correspondence—it does not signify that a function necessarily entirely lapses in the absence of a certain structure. All our observations indicate that the relatively few functions of the living substance—irritability, contractility, metabolism and reproduction—are shared by all its parts in varying degrees, and when we speak of the function of any somatic organ or cell part we mean its outstanding and preponderant activity.

With this understanding of the relation between morphology and physiology, we speak of the chromosomes as having to do with the process of reproduction, and conceive of them as non-homogeneous within themselves and individually unlike. In this sense they bear factors, or have parts, which are most concerned with certain peculiarities which, through cumulative action in cell reproduction, come to fullest expression in regions of the complete soma. Through what manner a factor operates to produce a cell structure which, in a given cell aggregate, summates in the form a somatic character, we do not know, and that is not the question immediately at issue in the individuality hypothesis. What is postulated there is that the chromosomes are self perpetuating entities with individual peculiarities of form and function to identify them. Characteristics of form and behavior we see; certain very definite parallels between these and the manifestations of somatic characters exist beyond question; provision for the perpetuation of the organic unity of the individual chromosomes is found in the process of mitosis; the actual direct result of its operation appears in the uniform conditions of the complex in the individual animal; the extension of this beyond the organism to the group and the means for it in the phenomena of maturation and fertilization are easily established by observation; the age old existence of all these circumstances is revealed by the near approach to uniformity in the chromosome complex of the multitude of species of unnumbered individuals constituting a family. And yet, in the face of this overwhelming mass of evidence indicative of order, system and specific chromosome organization, some conceive only the action of ordinary chemical forces, or the chance association of indifferent substances, while others, over impressed with the thought of a general coordinating force in the organism, deny significance to the orderly play of its cellular parts.

While opponents of the individuality hypothesis differ thus widely in what they would substitute for it, they almost invariably seize upon one supposed condition of the chromosome as a basis for discounting the remaining positive evidence which is presented for the hypothesis. Even some well informed cytologists who accept the implications of the facts, regard this circumstance as a severe weakness in the chain of evidence. That the chromosomes do not maintain a compact and easily recognizable form in the interval between mitoses is accepted by many such biologists as proof that they no longer exist as entities. All the other manifold indications of character and continuity do not weigh against this apparent loss of identity. Doubtless it would be more satisfying if we could at all times perceive the chromosomes in unchanging form in all stages of cellular activity, but why we should demand this condition as a test for individuality in the chromosomes when we unhesitatingly admit the unity of an organism in all the varied changes of its development from a single cell, through such complexities of change and metamorphosis as to give rise to doubts of even the phyletic position of some stages, it is difficult to see. Being organic, the chromosomes must change their form, they must suffer division of their substance and they are obliged to restore this loss through metabolic changes. Since these changes of substance take place at surface contacts there is an obvious advantage in increased superficies and, in common with other, larger structural elements, the chromosomes become extended and their substances are diffused. In this state their boundaries may not be well defined and this circumstance has been seized upon as a disproof of their continuity.

For myself, even were the visible limits of the chromosome completely lost, this would not appear as a convincing disproof of persistent individuality in the face of the large number of facts pointing in the opposite direction. Of such facts those relating to the history of the multiple chromosomes, outlined in this paper, are of great importance. Their bearing upon the various aspects of numbers, sizes, forms and behavior, has been mentioned under these different headings. At this point however I wish to summarize this evidence upon the general topic of individuality. But before doing so I should like to repeat statements previously made that the accessory chromosome in the germ cells of the male presents us with the history of a particular chromosome, at all times distinct and well delimited, whose physical identity suffers no eclipse during the various metabolic changes of growth and division. It is readily distinguished where it occurs, not alone in a given cell, but in all the germ cells of the species, genus, family and order. The same element, always recognizable by reason of its structural character, is identified in different phyla and is shown to have a definite relation to the development of sex characters. It would seem impossible to conceive any more definite marks of individuality than is possessed by this chromosome, in whose history there is no confusion of character or break of continuity, and yet it does not receive even mention in some attacks upon the theory of individuality.

Again, its position may be granted, but with the reservation that it is unique and no criterion for other chromosomes. Upon this point it is now merely necessary to say that the accessory chromosome differs only in degree from the euchromosomes, and general conclusions regarding the nature of chromosome organization may properly be drawn from its history. That its condition is indeed not unique is shown by various approaches to it on the part of certain chromosomes in Phrynotettix, as demonstrated by Wenrich ('16). The great similarity to the euchromosomes is also indicated by the union with the accessory chromosome in multiples, and by its entirely typical nature in the female when paired.

The mere academic question of individuality is not here important, the practical matter before us is to decide whether the metaphase chromosomes of two cells are individually identical organic members of a series because they were produced by the observed reproduction of a similar series of the parent cell, or whether the resemblance is independent of this genetic relation and due to chance association of indifferent materials, or to a reconstituting action of the cell as a whole. It is my belief that the observed act of reproduction, by which the organization of the chromosomes is materially transmitted in each mitosis, together with all facts indicating extensive distribution of given conditions, definiteness of organization, uniformity of behavior and consistency of deviation from the normal, are so many clear indications of the individual character of the chromosomes. Transmutation of form, even to an extreme degree, can not be held as a valid argument against a persistent individuality. A consideration of the criteria applied to larger organic aggregates well supports this view. Such objects are said to possess individuality when they exhibit a more or less definite unity which is persistent and characterized by peculiarities of form and function. Most clearly defined is this individuality when it may be perpetuated through some form of reproduction to find expression in new units of similar character. The term does not connote unchangeability, and there may be fusions with more or less loss of physical delimitations, followed by separation, even after exchange of substances. The test of individuality is material continuity, but it does not necessarily involve complete or entirely persistent contiguity. An organism may bud off new individuals similar to itself, the substance of its body differs from time to time, movements of parts take place, fragmentation occurs, extreme attenuation or extension of substance is found, even separation and recombination of parts may happen and yet the individual maintains itself. What it may have been in the past, what its possibilities of future development are, what potentialities of multiplied individuality it suppresses do not affect the reality of its individuality. It is, as Huxley says, "a single thing of a given kind." If one such thing divides into two, there are two individuals; if two unite into one indistinguishably there is a single individual; if a fusion of two things occurs in part, without loss of physical configuration, there are still two individuals in existence. Only when the substance of one thing disappears or becomes incorporated integrally into the organization of another does its individuality depart.

If all these variations of physical state may occur in the history of an organism without sacrifice of individuality, there can be no reason for urging them against a conception of the individuality of the self perpetuating chromosomes. Especially is this true in face of the facts recorded here and in other papers showing the high degree of chromosome constancy, for any given period, in all the attributes by which we usually judge individuality. The conditions in Hesperotetix and Mermiria at first seem to be a contradiction to this generalization, but, when examined with care, show, not an instance of fundamental change, but merely of modified detail. From these results it is very clear that a chromosome number less than normal for the group is not a necessary evidence of lost elements. In a similar manner the studies of Miss Holt on the intestinal cells of Culex indicate definitely that numbers in excess of the normal do not signify the addition of anything not previously represented. Loss or gain in chromosome numbers is a condition to be investigated, not implicit evidence of an altered organization with loss of organic individualities. That the chromosomes are not the ultimate structural units is, however, indicated in many ways, but it remained for Wenrich to show the nature of this more minute portion of the chromosome architecture. From his results it would appear that all chromosomes are, in a sense, multiples, and it is conceivable that these parts might be variously associated, producing even extensive numerical variation, still without loss or gain of essential structural elements.

The peculiar case of Ascaris megalocephala, where evident multiple chromosomes are retained intact in the germ cells while they suffer fragmentation in the somatic cells, is an extreme instance of numerical variation which occurs in one individual. How common such relations may be we do not know, since so little has been done in the study of the complete history of a single organism; but it is obvious that the Orthoptera stand at the opposite extreme from Ascaris in the persistence of chromosome organization, for here all the cells of an individual seem to be invariably the same, and the individual is representative of the group. But even in this stable assemblage we encounter the conditions which I have described for H. viridis. This case, with its diminution in number, and that of Culex, with its great increase, are alike in the fact that the measures of difference are entire chromosomes and not parts of such, as seems to be the case in Ascaris, unless indeed these chromosomes be multiples of a high order of complexity.

If it were possible for chromosomes to reproduce themselves and still preserve their physical configuration unchanged, there would probably be little question of their continuity and individuality—the demonstration would be self evident. But it happens that the necessities of the case require that each newly produced chromosome should take part in the formation of a new nucleus, through whose activities the cell as a whole and each chromosome, individually, is enabled to restore the volume diminished by the act of division. During this process the outlines of the chromosomes become materially changed and in their extreme diffusion can no longer be traced in many cases. Because of our limitations in observational power they appear to be lost as separate individuals and we are thus deprived of the simple test of observed continuity. Later, in the same cell, there reappears a series of chromosomes severally like those which seemed to disappear during the period of metabolic activity.

We confront two alternative explanations for this re-integration of the chromosomes; either they actually persist as discrete units of extremely variable form, or they are entirely lost as individual entities and are reconstituted by some extrinsic agency. There is no other possible explanation and we must weigh the evidence for one or the other of the alternatives.

All facts which indicate order and system in chromosome features speak for the former, those which demonstrate variability and indefiniteness, for the latter. The case for discontinuity is strongest in the absence of any chromosome order, and becomes progressively weaker with the establishment of definiteness and precision in form and behavior. Evidence has already been given in other papers on the Orthoptera to show the exactness in organization of chromosomes, their persistent continuity between mitoses in certain cases, the uniformity in number and behavior through large groups, and the parallel behavior of chromosomes on one hand, and of somatic characters on the other. In this paper are discussed what appear to be the widest departures from normal conditions so far found in the group. Accepting as most important, next to actual observed continuity, the evidence afforded by constancy in number, size, form and behavior of the chromosome, I have been able to show that these criteria are here applicable and that variation is only apparent and not real. It seems to me that the facts disclosed in Hesperotettix and Mermiria not only fail to weaken the case against individuality, as tested by constancy in number, size, etc., of the chromosomes, but, on the contrary, greatly strengthen it, for the reason that all of the distinguishing features of the individual chromosomes are maintained, even in the presence of unions into multiples of higher order. The fact that, although the number of independent units is reduced, nothing is lost from the complex, is most important and significant.

Finally, the main feature of chromosome individuality must not be lost in a discussion of whether it is the chromatin or the linin that persists with less change. However great in importance this distinction may be, it does not alter the aspect of the problem of individuality in its present phase. One or both of these substances may be responsible for the integration of the chromosomes, but it is the fact that there is a definite structure to be identified which concerns us most. The substances of the chromosome divide into new units of similar relative sizes, and these structures, now of half the original content, take up unlike materials and replace the missing portions. The complexes of the daughter cells are now equivalent to that of the mother unit. It is obvious that, since the chromosomes, as such, divide, all of their substances must be involved. There may be some question whether one may be lost as a morphological entity while another persists—although this is much open to question—but something maintains the organization which we recognize in the chromosome and it is this organization which we study. In view of the fact that our microchemical tests are so far from specific in their action, it is not the part of wisdom to build very extensive theories upon their evidence. It is quite possible that one substance, in different phases of its activity, may present alternately the aspects of chromatin and achromatin, and some conditions of the staining reaction suggest this, so that it would be unwise to involve the whole question of chromosome organization in a dispute regarding the nature of their substance, based merely upon uncertain staining reactions.

8. *Chromosome specificity*

That chromosomes might be genetically continuous and still be of the same nature, without differential character, is, of course, conceivable. In fact it is more than probable that, in a sense, they are all alike, for they probably share the general properties or functions of protoplasm. But that they carry out these activities in the same manner is a conclusion quite foreign to the evidence. Just what the nature of this differential action may be has not yet been discovered, but the action of the sex determining chromosome and the groupings of characters in Drosophila are suggestive. Male and female possess the same series of parts, and the difference between them is one of relative development. Nothing unique for either sex exists and such a thing as an 'exclusively male' or exclusively female character is not to be found. The evidence here would indicate that the 'factors' for maleness, are such controls of the developmental processes as will eventuate in a certain degree of differentiation of each cell of the body. The sum of all these, in any somatic region, produces a condition which we call a male character, and the total complex of these constitutes an individual which is a male. In another individual the same series of elements obtains, but each cell and each part is slightly different and the sum total of the characters produces an assemblage which we recognize as a female. Should the conditions be varied, even by the internal secretion of the gonad, in some cases, these may be altered to resemblance of the opposite sex.

However much the entire complex is involved in the production of characters which are called sexual, it is apparent from the history of the accessory chromosome in the Orthoptera that it is a differential agent. All other things apparently being equal, the presence of one accessory chromosome so shapes the developmental processes that a male results, while if two are involved a female is produced. In some way, not now apparent, the action of this particular one of the chromosomes differs from all the others in producing an effect of which they are not capable—that is, it has a specific action. Apparently it is unlike them in being concerned with the entire body, including the germ cells, which are differentiated into eggs or spermatozoa. A further indication of the specific nature of this particular chromosome is afforded by sex-linked characters whose development is conditioned by exactly the same circumstances of distribution as those which mark the alternatives of maleness or femaleness. The facts relating to the accessory chromosome are the strongest evidence we have in support of chromosome specificity, because the history of this element is so clear, its continuity so unbroken and its relations to certain characters so definite. But any evidence for specificity of one chromosome is, at the same time, support for the general conception of the differential nature of chromosomes, and for this reason the facts concerning the accessory chromosome have additional value.

The only direct evidence for any such a differential nature of the other chromosomes is afforded by the work on Drosophila. In this case a whole group of characters follows the rule of distribution governing sex. This is the only element that can certainly be identified, but the groups of observed characters, not sex linked, correspond to the three remaining pairs of chromosomes, and the numbers of characters in each group are in proportion to the size of the chromosomes. Of these one pair is very small and there is a corresponding small group of characters. As is well known, the analysis of the conditions in Drosophila has gone so far that the relative loci for the different factors have been calculated. It would seem from these results that there is every reason to regard the chromosomes of this fly as qualitatively different. While there is no correlation of this sort known for the Orthoptera, the actual history of the chromosomes which their germ cells exhibit affords a mechanism adequate for the facts of alternative inheritance and for the segregation and chance recombinations of characters. While the multiple chromosomes do not afford any direct evidence for specificity of function, it would seem, because of all the facts, that the purpose for which these studies on the Orthoptera were begun—a correlation between germ cell structure and somatic characters—is much more feasible of accomplishment than appeared at the beginning.

V. SUMMARY OF RESULTS

1. Chromosomes are definitely organized chromatic bodies acting as units in mitosis.
2. These units are of unlike morphological value in the different generations of germ cells.
3. While any one cell generation is marked by one general type of chromosome organization, individual chromosomes may differ from the type in higher or lower degree, by definite steps or intervals.
4. In a given species the integration of the chromosome complex may vary from individual to individual.
5. For any one individual this integration is fixed.

6. Despite this individual variation in the composition of the mitotic units, there is no loss in the total morphological elements of the specific complex, or departure from the usual habits of synapsis and segregation of homologous elements.

7. The maintenance of the original complex in the presence of a variable number of mitotic units results from associations of these to produce others of higher valence.

8. Where variation between individuals exists for any one cell generation, it is the result of differences in association between certain definite mitotic units.

9. Such variations are not indefinite, but occur in a fixed order between certain units.

10. The occurrence of associations between units of lower order into those of a higher results from the operation of a common integration principle.

11. The union of homologous chromosomes to produce tetrads is temporary and is terminated in one of the following maturation divisions; multiples of non-homologous chromosomes are permanent for the individual and the union does not end in either maturation mitosis.

12. Associations in the first spermatocyte of a higher order than tetrads are the result of lower associations which are persistent throughout all cells of the individual.

13. Associations into tetrads in the first spermatocyte occur between homologous chromosomes.

14. Higher associations than tetrads in the first spermatocyte are necessarily non-homologous.

15. Associations may be complete and persistent, or only approximations.

16. Associations may be complete in parts of chromosomes and incomplete in other regions.

17. The point of union between associated units in metaphase is at the ends of the chromosomes.

18. In telomitic chromosomes this is at the point of fiber attachment.

19. Atelomitic chromosomes of unit value have not yet been observed in multiples, except in that part of the tetrad of Mermiria not joined to the accessory chromosomes.

20. Multiple chromosomes are of two types (a) those between euchromosomes and heterochromosomes and (b) those between euchromosomes alone.

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DESCRIPTION OF PLATES

All figures are drawn at an initial magnification of 2360 diameters under the camera lucida, and appear in the reproduction at a magnification of 1800. The photomicrographs were obtained with a Zeiss 2 mm. objective of 1.40 N. A., projection ocular 4 and a Watson 'Holoscopic' oil immersion condenser. The original magnification of 1000 diameters is here reduced to 666, except fig. IX.

PLATE 1

EXPLANATION OF FIGURES

Horizontal rows represent complete first spermatocyte complexes, usually in lateral view, numbered 1 to 6. These are arranged so that chromosomes of homologous size are brought into vertical rows, numbered from 1 to 12. Blank spaces in vertical rows represent the normal position of the accessory chromosome which is joined to a tetrad.

1 A complex of Hesperotettix brevipennis. All twelve chromosomes are free.

2 Hesperotettix festivus, polar view.

3 Hesperotettix viridis, class 1—twelve chromosomes plus a supernumerary.

4 H. viridis, class 2.

5 Hesperotettix pratensis.

6 Hesperotettix speciosus.

MULTIPLE CHROMOSOMES

CLARENCE E. MCCLUNG

PLATE 1

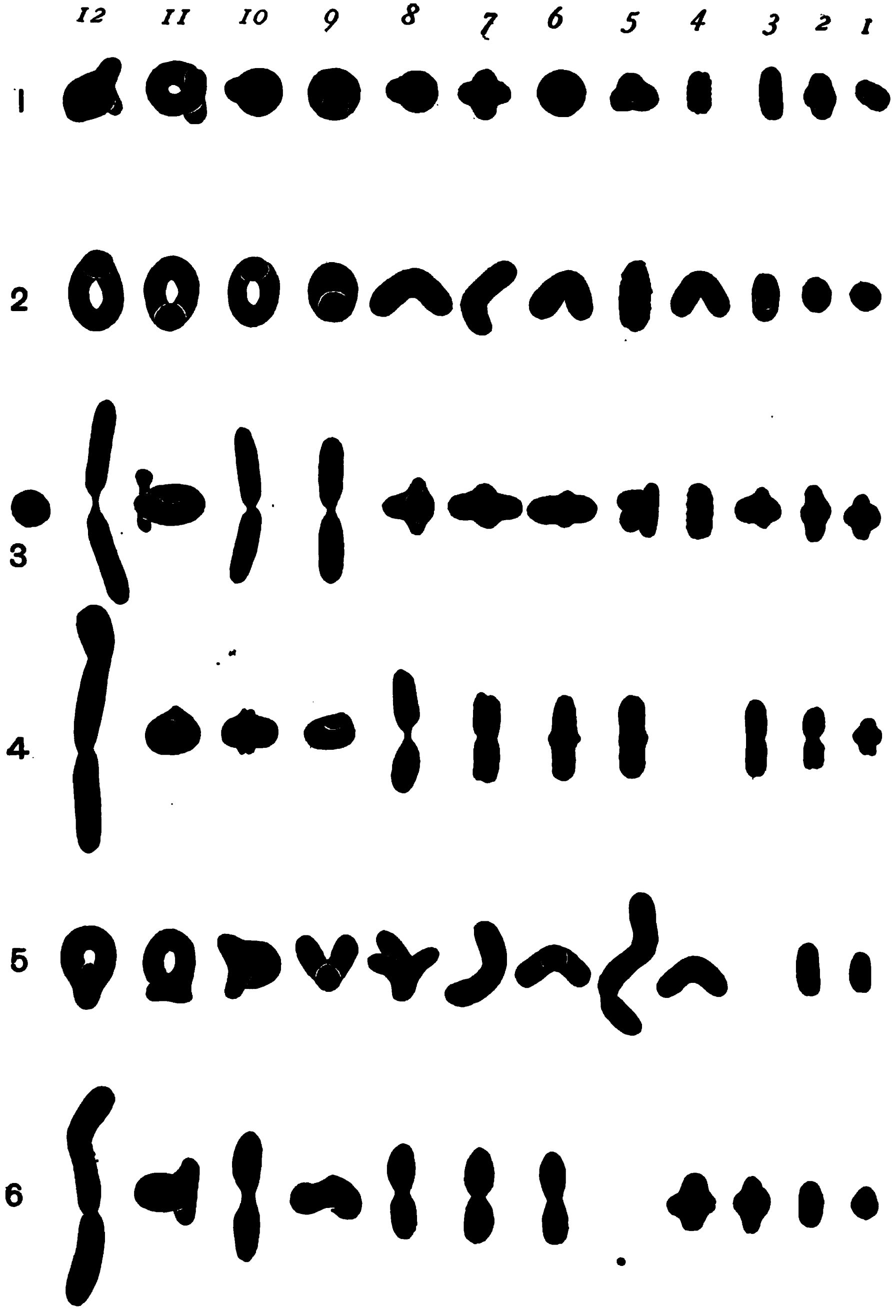


PLATE 2

EXPLANATION OF FIGURES

Complexes, arranged as in plate 1, of representatives of the various classes of first spermatocytes in Hesperotettix viridis. The classes are indicated by the figures at the left, 1 to 6. Euchromosome multiples are arranged between the rows to which their tetrads belong. Not all conditions are represented here but may be found in other figures. Thus the smaller octad of class 4 may also be a ring in some individuals; the smaller ring of class 5 may be a V; and the ring octad in one individual of class 6 is a V.

MULTIPLE CHROMOSOMES
CLARENCE E. McCLUNG

PLATE 2

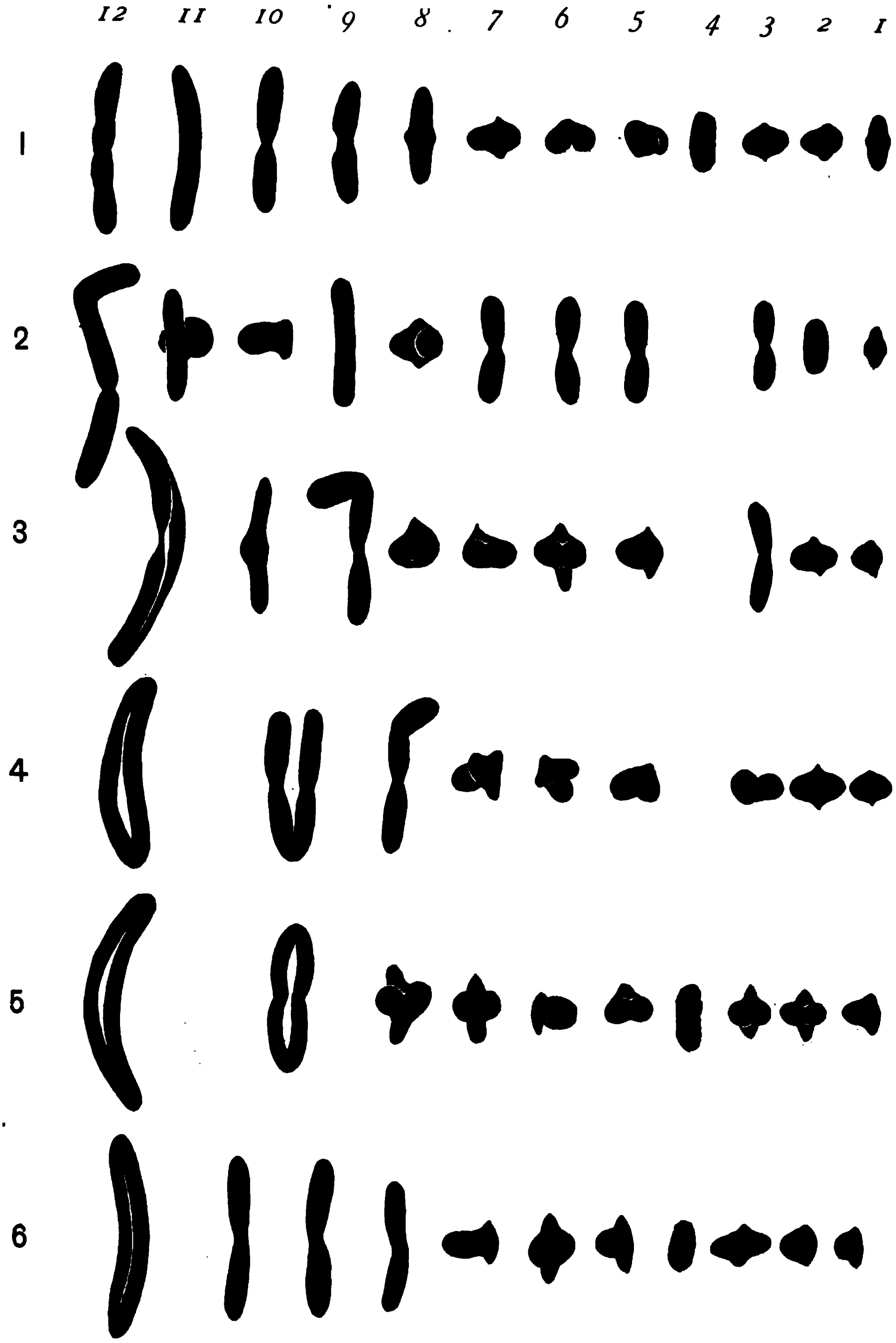


PLATE 3

EXPLANATION OF FIGURES

1 to 5, 7 to 9 Polar views of spermatogonial metaphase complexes. 1 to 7 are of Hesperotettix viridis, 8 of H. brevipennis and 9 of H. speciosus.

1, is of class 2, showing twenty-two chromosomes, of which one is a multiple. The accessory chromosome portion of this is marked '*X*.'

2 An example of class 3. The members of the large first spermatocyte euchromosome multiple ring are the larger V-shaped elements, while the smaller V includes the accessory portion of the hexad. Twenty separate chromosomes.

3 A complex of class 4, in which the largest two V's are parts of the first spermatocyte ring, the next largest one of the octad V, and the smallest of the hexad. Nineteen separate chromosomes.

4 In this group, representing class 5, there are three V's belonging to two octads of the first spermatocyte, the smaller of which is a V. The accessory chromosome is free. Twenty separate chromosomes.

5 From an individual of class 6 with a free accessory chromosome and one octad ring in the first spermatocyte. Twenty-one chromosomes.

6 An anaphase group of class 4 showing the 4 V's (fig. 3 above).

7 A complex of class 6 in which the octad of the first spermatocyte is a V instead of a ring. The one V in this spermatogonial group is involved in the formation of this multiple. Twenty-two free chromosomes.

8 From H. brevipennis in which all specimens studied showed twenty-three rod shaped chromosomes.

9 The one V in this complex of twenty-two chromosomes will constitute a part of the hexad in the first spermatocyte. The accessory chromosome forms one limb.

10 An octad ring of H. viridis in the late prophase. It will commence to separate at the apex.

11 Late prophase condition of such a chromosome as shown in figure 10.

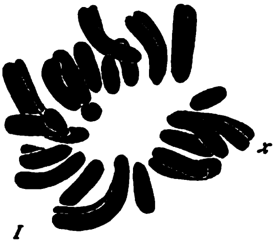
12 Shows metaphase condition of such octad rings. These are from different cells.

13 Two metaphase octad rings of H. viridis from the same cell. Class 5.

14 Similar elements from another cell of the same individual as shown in figure 13. The large octad *b* has one tetrad forming a ring lying in the equatorial plate at right angles to the other member. The small octad *a* shown edgewise.

15 Five examples of the V-shaped octad of class 4. The inequality of the constituent tetrads shows clearly.

16 Multiples of class 4; *a* the large octad and the hexad; *c* the two octads, the larger like figure 14 *b*.



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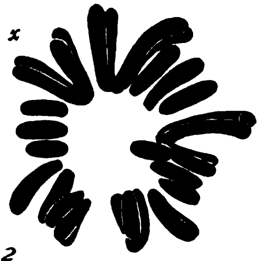

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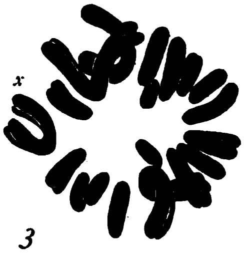

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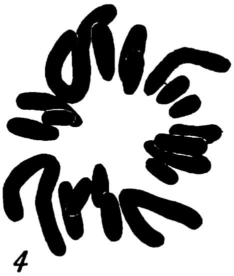
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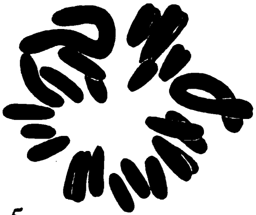
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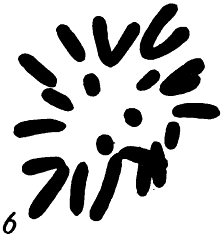
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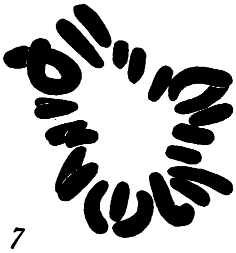
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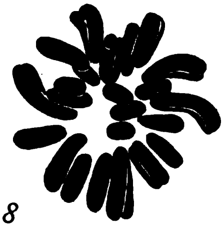
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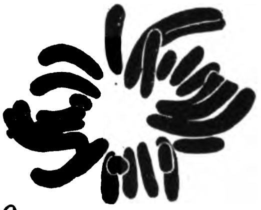
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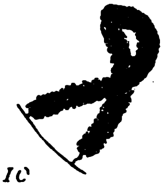
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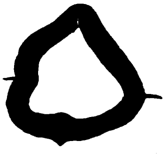
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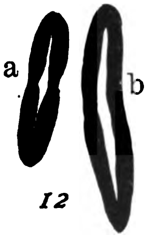

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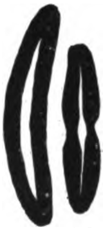
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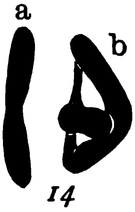

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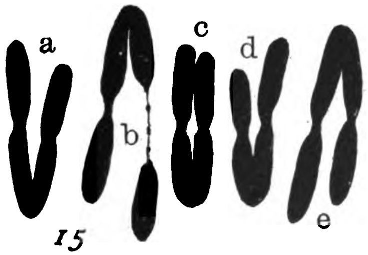

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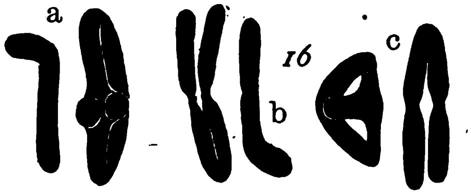

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PLATE 4

EXPLANATION OF FIGURES

17 Examples of octad rings in late prophase showing various aspects during the process of opening out. These are arranged somewhat in the order of progress from *a* to *k*.

18 Lateral view of first spermatocyte metaphase, class 6. One octad ring, and accessory chromosome at one pole.

19 Three examples of extended V's, such as shown in Photo Y.

20 Lateral view of first spermatocyte metaphase of class 5 with the V portion of the smaller octad going to same pole as the accessory chromosome. Ten free chromosomes.

21 A complex of a first spermatocyte of class 6 in which the one octad is a V.

22 Two first spermatocyte octads from one cell.

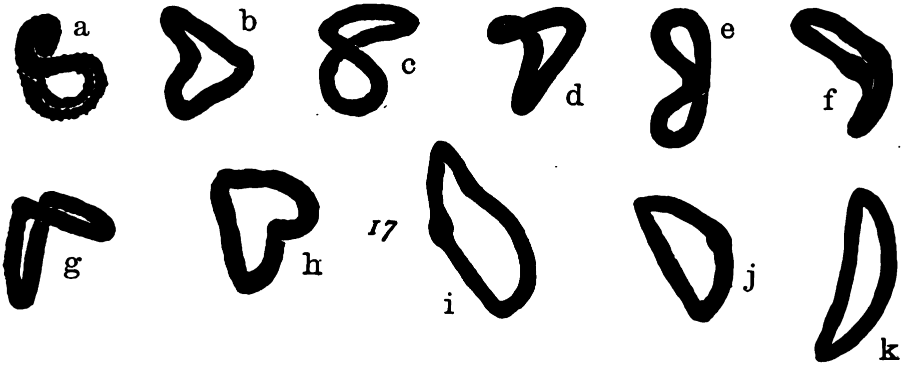
23 A complex of H. pratensis in which the hexad is similar in shape to that of Anabrus.

24 A complete complex of Hesperotettix viridis, class 3, in which the octad has opened at one side, simulating the appearance of the hexad in Mermiria.

25 The three multiples from a complex of class 4.

26 An entire complex of class 3, from the same animal as in figure 24.

27 A representative complex of class 5 with one V.



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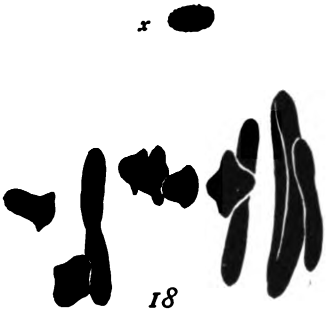

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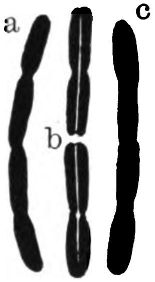

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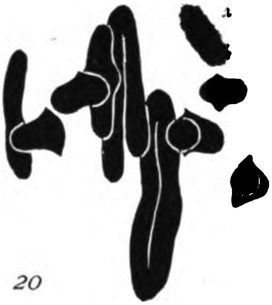

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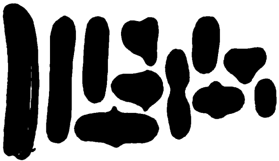

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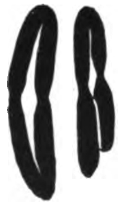

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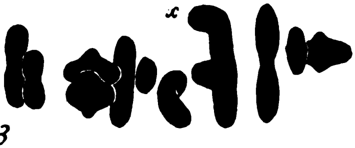

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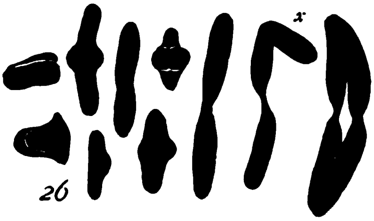

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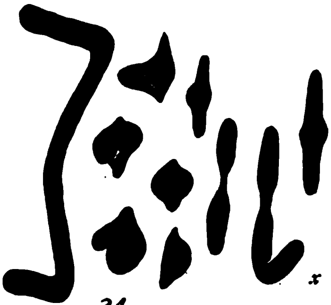

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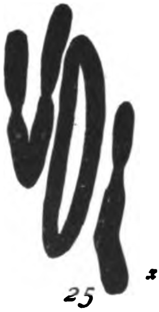

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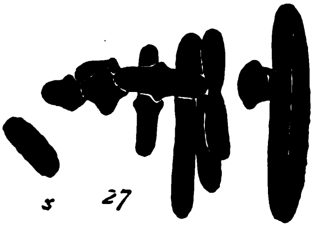

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PLATE 5

EXPLANATION OF FIGURES

28 First spermatocyte anaphase, class 4 with one V, showing the distribution of the rod and V parts, in black. The entire complex, including the other multiples is shown.

29 to 42 Various second spermatocyte metaphase complexes (except fig. 33) in polar view, of H. viridis.

29 Class 1 with accessory chromosome.

30 Class 2 without accessory chromosome.

31 Class 2 with accessory chromosome in multiple.

32 Class 4, (one ring, one V and hexad in first spermatocyte) no accessory chromosome, two rods of V, and half of ring.

33 First spermatocyte anaphase of class 4, no accessory chromosome.

34 Class 6 (in first spermatocyte one ring, free accessory chromosome) no accessory chromosome.

35 Class 6 with accessory chromosome.

36 Same condition as figure 35, same animal.

37 Same condition as figure 35, another animal.

38 to 42 From one animal of class 6 with the octad in the form of a V, accessory chromosome free.

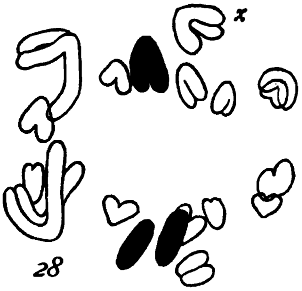
38 With V and no accessory chromosome—ten chromosomes.

39 With V and with accessory chromosome—eleven chromosomes.

40 No V and no accessory chromosome—eleven chromosomes.

41 As in figure 40.

42 No V, but with accessory chromosome—twelve chromosomes.



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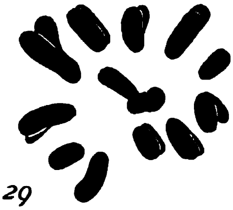

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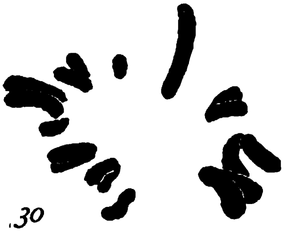

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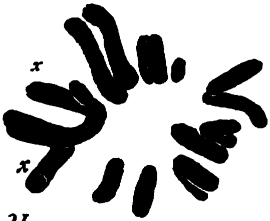

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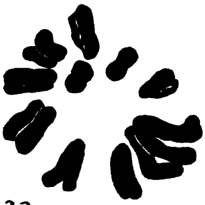

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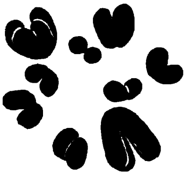

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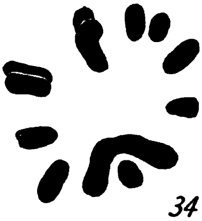

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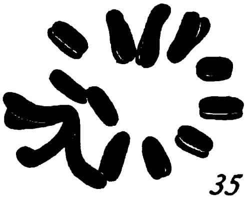

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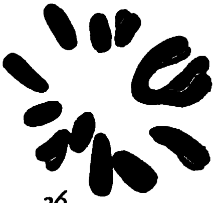

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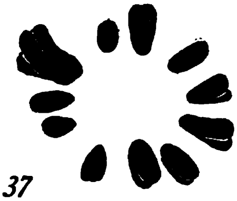

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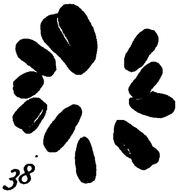

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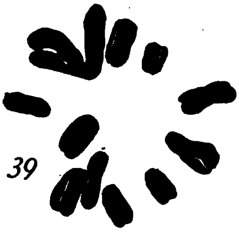

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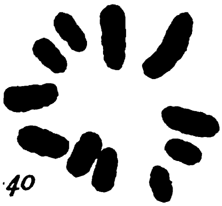

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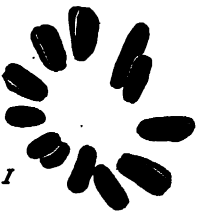

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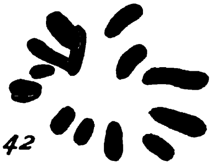

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PLATE 6

EXPLANATION OF FIGURES

43 to 54 From Mermiria bivittata.

43 Somatic complex of the female with twenty-two free chromosomes and two similar multiples, each containing an accessory chromosome. The euchromosomes are all telomitic.

44 A spermatogonial complex of twenty-two free chromosomes with two unlike V's, in one of which the accessory chromosome betrays its presence by its characteristic granular condition. The other V is smaller and is confined to the male line by always going into the opposite cell from the accessory chromosome in its segregation division.

45 Another female somatic complex.

46 Spermatogonial complex.

47 Pairs of spermatogonial V's in different stages showing disproportion in size.

48 A female somatic complex—one V foreshortened.

49 Spermatogonium, V-chromosomes. *a* and *c* associated pairs.

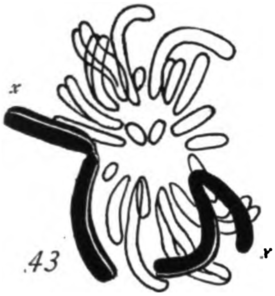
50 *a* The multiple in first spermatocyte anaphase, lateral view; *b*, *en face;* *c*, as these elements would appear in second spermatocyte metaphase.

51 Multiples in late first spermatocyte prophase showing the marked difference in appearance between the smooth, homogeneous, condensed accessory chromosome and the yet granular tetrad to which it is joined.

52 First spermatocyte multiples, *b* almost fully condensed and showing non-chromatic clefts. These are about in the relative positions of the fiber attachments and the plane of division.

53 As in figure 51.

54 Like 52 *b*.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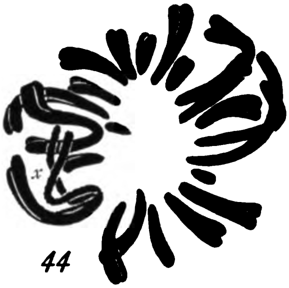
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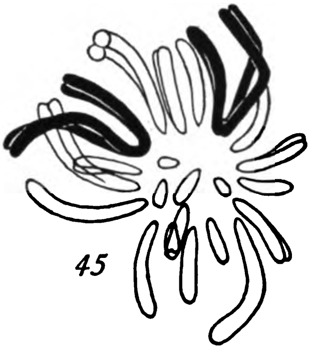
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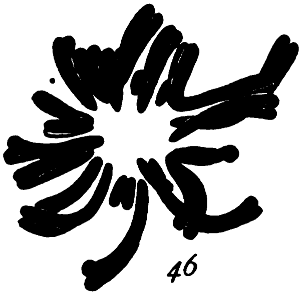
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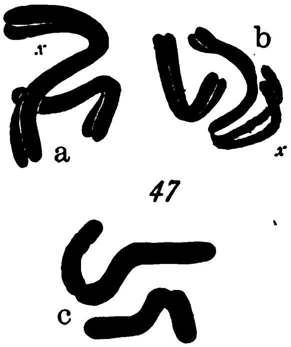
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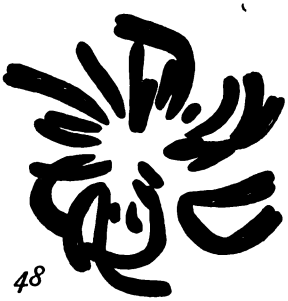
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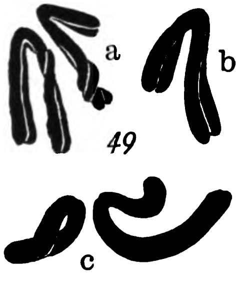
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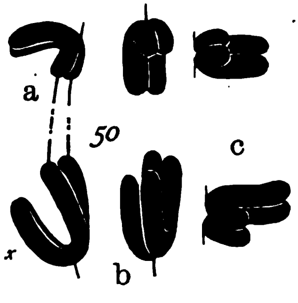
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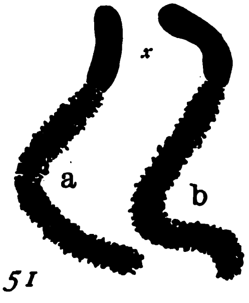
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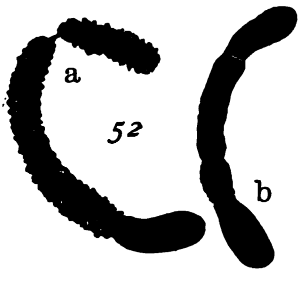
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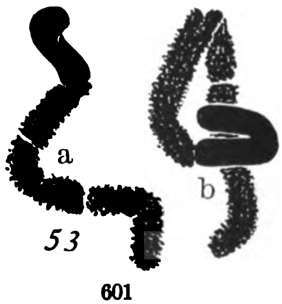
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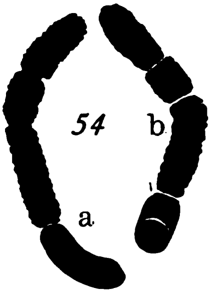
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PLATE 7

EXPLANATION OF FIGURES

55 to 65 From Mermiria bivittata.

55 First spermatocyte metaphase showing the multiple and the five smaller chromosomes divided.

56 Three multiples of first spermatocyte metaphase.

57 Like figure 56.

58 Three multiples of the 'green' variety of M. bivittata in which fiber attachment is almost at the end of the bent portion. At *a* complete separation, in *b* and *c* beginning stages.

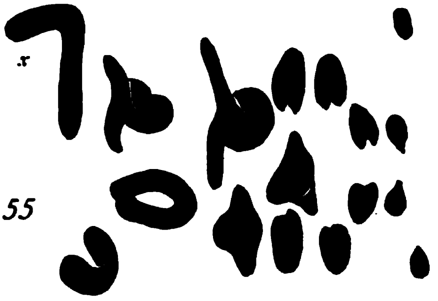
59 Similar to figure 58. Note the various constrictions, simulating a decad constitution, emphasized at *a* by separation at two levels.

60 Complete separation of bent rod opposite accessory chromosome.

61 Polar view of second spermatocyte metaphase.

62 Lateral view of same stage.

63 to 65 Polar view of second spermatocyte metaphase. All have eleven chromosomes, one of which is a V, but in one case, figure 63, this is a simple chromosome and in the other, figure 64, it is a multiple involving the accessory chromosome.



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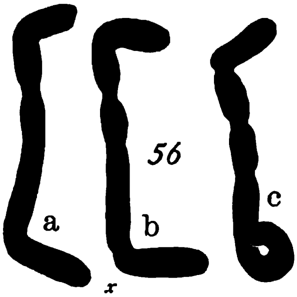

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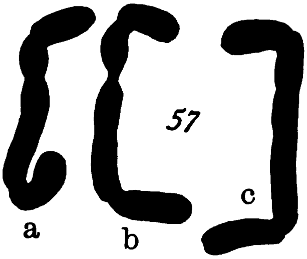

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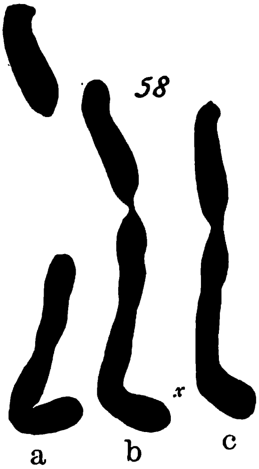

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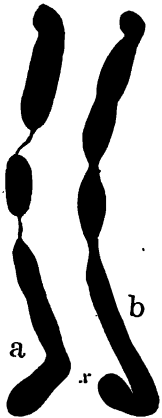

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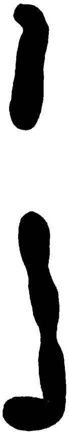

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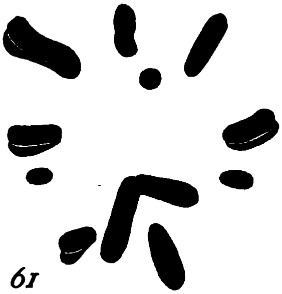

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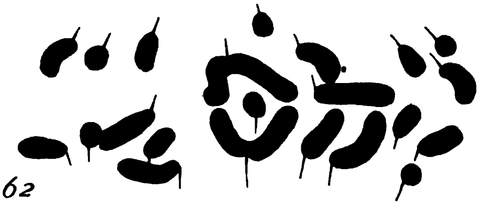

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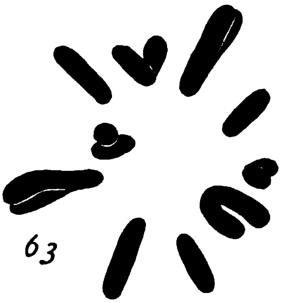

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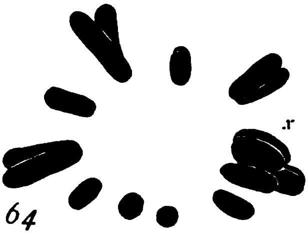

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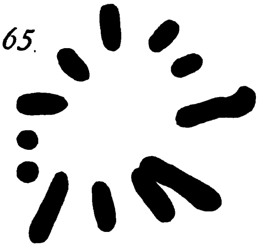

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PLATE 8

EXPLANATION OF FIGURES

A to Z and II to III are photo micrographs of Hesperotettix viridis cells and IV to IX of Mermiria bivittata. A to D and IV to VI smears, the remainder sections.

A, B, C show the appearance of multiple rings with the longitudinal split and numerous clefts, D is a lateral view of a first spermatocyte metaphase showing the multiple ring in profile and the accessory chromosome at one pole.

E Polar views of spermatogonial metaphase with telomitic and atelomitic chromosomes.

F, G, H Profile views of hexad multiple.

I to N Lateral views of V-shaped multiples showing characteristics of form and movement.

O Ring and V. multiples.

P, A V-multiple with one limb out of position.

Q to T Ring multiples in which one tetrad has the plane of its ring perpendicular to the other.

U, V Late stages in the movement of such rings as appear in Q to T.

W, X Late metaphase stage of ring multiples.

Y An extended V similar to that of P.

Z A ring multiple about to divide.

II Anaphase condition of a V-multiple (cf. fig. 28).

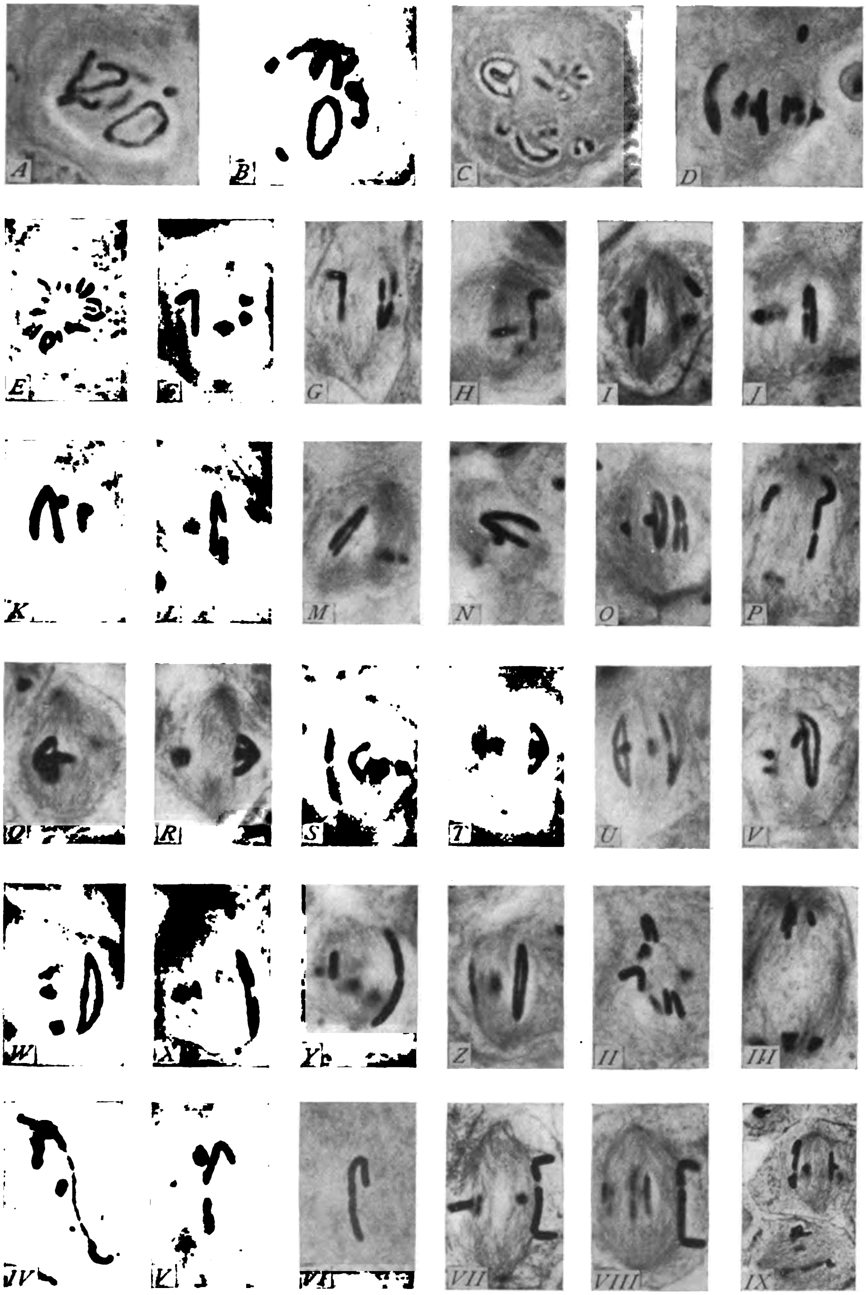
III Late anaphase condition of a divided ring. The resulting V's are double.

IV Prophase condition of a hexad showing the accessory chromosome condensed and the tetrad thin and granular.

V, VI Metaphase hexads showing constrictions.

VII, VIII Profile views of hexads.

IX Lateral views of mitoses × 400.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SYNAPSIS AND CHROMOSOME ORGANIZATION IN CHORTHIPPUS (STENOBOTHRUS) CURTIPENNIS AND TRIMEROTROPIS SUFFUSA (ORTHOPTERA)

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Department of Zoology, University of Pennsylvania

THREE PLATES

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I. INTRODUCTION

In a recent paper (Wenrich, '16) the writer presented what seemed to him conclusive evidence that parasynapsis occurs in at least three pairs of first spermatocyte chromosomes of Phrynotettix magnus. Although only these three chromosomes were studied in great detail, sufficient attention was given the other members of the complex to assure the writer that parasynapsis occurs in all. Phrynotettix, however, like many of the Oedipodinae, possesses only rod-shaped, or telomitic chromosomes. It seemed advisable, therefore, to investigate the conditions to be found in species of grasshoppers in which the chromosomes are V-shaped, or atelomitic. Chorthippus (Stenobothrus) curtipennis was chosen for this study because it is readily obtainable, gives clear figures when fixed and stained, and because various species of the genus have been the subject of investigation in the past. Sufficient study of this species, which has three pairs of atelomitic chromosomes, was made before the publication of the paper on Phrynotettix to assure the writer that the chromosome relationships in synapsis are essentially the same as in that form with respect to both types of chromosomes. This is indicated by the following statement (Wenrich, '16, p. 98):

> I have recently made a study of the conditions in Stenobothrus and may say that I found parasynapsis for both forms of chromosomes, and that the V-shaped chromosomes divide reductionally in the first maturation mitosis as Davis ('08) described, but that the rod-shaped chromosomes divide equationally in the first division as I found that they did in Phrynotettix.

No drawings were presented to support these statements at the time, but the evidence (with additions) on which the conclusions as to parasynapsis were based is presented in the present paper. Unfortunately, more careful study of the material makes it impossible to be so confident as to which division is reductional for the two types of chromosomes. This point will be discussed later.

The same conclusions as to parasynapsis in Chorthippus, together with the same kind of evidence, in part, has recently been presented by Robertson ('16). In many respects the evidence and conclusions which have been independently reached by Robertson and myself will be mutually corroborative. In regard to some points, however, differences of interpretation exist which will be discussed in an appropriate place.

In view of the very interesting conditions in Trimerotropis and Circotettix, where telomitic chromosomes are paired with atelomitic ones, as found by Dr. E. Eleanor Carothers ('17) it seemed worth while to examine stages which would indicate what form of synapsis occurs between these chromosomes of diverse form. Dr. Carothers very kindly permitted the use of her slides for this purpose. It may be stated at once that parasynapsis was found to occur for all the chromosomes of Trimerotropis of whatever form, just as it does in Chorthippus and Phrynotettix. In addition to the subject of synapsis the topics of pre- and post-reduction and chromosome organization will be considered.

In this paper new terms will be used in accordance with those recently adopted by Dr. Carothers ('17), as follows: (1) *Telomitic*—chromosome with terminal fiber-attachment, i.e., rod-shaped; telomitic and rod-shaped will therefore be used interchangeably; (2) *atelomitic*—chromosome with non-terminal fiber-attachment = V-shaped; V-shaped and atelomitic will be used interchangeably; (3) *heteromorphic*—those pairs of chromosomes (tetrads) of which one member is telomitic and the other atelomitic, or, of which one member is noticeably different in form or size from its mate; (4) *chromomere-vesicle*—the plasmosome-like appendages formerly called 'vesicles' by Carothers ('13), and called appendages and plasmosome-like structures by the writer ('16). I shall also make use of McClung's ('14) term, *euchromosome* as equivalent to Montgomery's 'autosome.'

All drawings have been outlined with a pencil under the camera lucida and then inked while the cell remained under observation.

II. OBSERVATIONS

A. Chorthippus (Stenobothrus) curtipennis

a. Introductory statement. Chorthippus (Stenobothrus) curtipennis belongs to the sub-family Truxalinae of the Orthopteran family Acrididae. The material from which nearly all the drawings were made consists of a single smear prepared during the summer of 1914 at Woods Hole, Mass., near which place this species was abundant. Through the kindness of Professor McClung I was afforded facilities for preparing and staining cytological material at that time. The smear was fixed in Flemming's stronger solution (at a temperature of about 4°C.). One part was stained with Zwaardemaker's safranin, the other with Heidenhain's haematoxylin. Figures 12, 13, and 14, plate 3, are from sectioned material, fixed in cold Flemming's fluid and stained with Heidenhain's haematoxylin.

While this material was studied some during the summer of 1914 and at Harvard University during the winter of 1914–1915, it was carefully re-examined in 1915–1916 at the University of Pennsylvania and again at Woods Hole during the summer of 1916, when most of the drawings were made. I am indebted to the University of Pennsylvania and to the director of the Marine Biological Laboratory for facilities for pursuing this study at Woods Hole.

Although material from a number of individuals was studied, nearly all the drawings are made from a single smear derived from one animal as stated above. It was deemed desirable to represent conditions from a single specimen in order to avoid any possible confusion arising through individual variations, since recent studies on such forms as Phrynotettix, Trimerotropis, Hesperotettix, etc., have shown that individuals within a species may vary as to certain features of their chromosomal constitution, but the conditions in each individual remain constant. In the second place, a smear was used in order to insure the presence of whole complexes in each cell and thus to avoid the difficulties incident to the study of sections, where most of the cells are cut and distributed into two or more sections with frequent disarrangements or cuts in the chromosomes. Some distortions naturally arise as a result of the smearing process, but the preparation used was more than usually free from such disturbances and in this case the advantage gained through the study of whole cells far outweighs the disadvantages arising from the smearing process.

In plate 1 all of the chromosomes in each cell are represented. It is believed, therefore, that, although identifications of any chromosome in any cell might sometimes prove doubtful, the chances for error, with all members present, is reduced to a minimum. This method also has its disadvantages, because only occasionally are all of the chromosomes in any particular cell in favorable positions for drawing.

b. The post-spireme chromosomes. The post-spireme stages have received the most attention and are represented on plate 1. As a glance at the plate will show, there are nine chromosomes in the reduced series. This number has been reported for all the species of the genus which have been studied except the doubtful member reported by McClung ('14) and which he now thinks may be Circotettix in which there were eleven. In plate 1, the chromosomes of eight cells are represented, those from each cell being arranged in a vertical column according to size, with the smallest at the bottom. The cells are lettered A, B, C, etc. Each horizontal row therefore presents examples of a particular chromosome, and successive stages in development from the end of the spireme stage to early anaphase can be read from left to right. These horizontal rows correspond in developmental stages to figures 62 to 65, plate 6, of the paper on Phrynotettix.

Of the nine chromosomes in the first spermatocyte one is the unpaired accessory chromosome (no. 3); the others are tetrads, consisting of three pairs of atelomitic (nos. 7, 8, and 9) and five pairs of telomitic chromosomes (nos. 1, 2, 4, 5, and 6). Column A represents the chromosomes of a cell at the stage when the spireme of the growth period has just become distinguishable as individual segments, each segment being the direct forerunner of a tetrad (except the acccessory, no. 3). Each of the segments is an attenuated thread with a longitudinal cleft, and more or less coiled or bent, according to its length. The longitudinal cleft is what has been called the primary longitudinal split.

In cell B, a second split is seen at some points, where four threads are visible (e.g., at *a*). In cell C all of the segments (except no. 1 and the accessory) have the four chromatids, indicating that the secondary longitudinal split has become complete. The other cells, D to H, represent the farther progress of chromatid transformation and condensation, through characteristic tetrad figures, to the metaphase or early anaphase of the first maturation mitosis. As nearly as possible, all of the tetrads in cells D to H have been given the same orientation; those in cells A, B, and C could not be definitely oriented, partly because the synaptic points could not be determined and, in the case of numbers 7, 8, and 9, partly because of lack of space on the plate.

c. Synapsis. The point to which especial attention should be directed is that all of the autosomes, or euchromosomes, show the same fundamental conditions, whether they be pairs of telomitic or atelomitic chromosomes. All come out of the spireme as elongated threads with one longitudinal cleft visible; all develop a second longitudinal cleft at right angles to the first, giving rise to tetrads, each with four chromatids. And since, as is generally believed, each tetrad represents a pair of spermatogonial chromosomes, it would appear that each of the spireme segments in cell A represents a pair of chromosomes which have been extended axially and united side-by-side throughout their length.

I have already shown ('16) how this side-by-side union of attenuated leptotene threads takes place in Phrynotettix. Evidence of the same nature for Chorthippus is presented in figures 1 to 4, plate 3. In figure 1 a nucleus is shown with one double thread (5)—that attached to the accessory—and another thread which is double at the end nearest the accessory (proximal end) but divides distally into two single threads. Numerous single threads are visible in the other parts of the nucleus. In figures 2 and 3 a considerable number of these partially conjugated pairs of threads are shown. In none of these drawings (figs. 1 to 4) was it possible to represent all of the threads because they are so long and so extensively curved and coiled that they appear at first glance to be hopelessly tangled and to constitute a network. It was possible, however, by careful study, to follow some of the threads for considerable distances, but others only short distances. On this account these drawings are, in part, diagrammatic.

In these lepto-zygotene nuclei there is always a tendency toward an orientation of the threads just as was found at the corresponding stage in Phrynotettix ('16, figs. 29, 30, 31, plate 3). This orientation appears to be confined mostly to the region near that end of the thread to which the spindle-fiber is attached (proximal end) and therefore that side of the nucleus toward which these ends are directed is called the proximal side. From this proximal side the threads run more or less parallel toward the opposite side of the nucleus, thence turning to traverse the nuclear space in various curved or convoluted courses. The amount of curving depends, apparently, on the length of the threads, though even the shorter ones have a tendency to form a loop as is indicated by the short, deeply staining thread (5), attached to the accessory chromosome in figures 1 and 4. In general, only the proximal ends of the threads exhibit orientation and this orientation, I believe, facilitates the initiation of the pairing process and causes its inauguration to occur at the proximal ends, as shown in figures 1 to 3. In the distal part of these figures most of the threads are single. The accessory (X), usually has one end attached at the proximal pole, as shown in figures 2 and 3. In figure 4 the position of the nucleus was such that the orientation could not well be shown without interfering with other features which it was desired to represent.

The nuclei in figures 2, 3, and 4 were chosen for drawing because they show, in addition to the partially conjugated threads already mentioned, certain others to which small plasmosome-like bodies, or chromomere-vesicles, were appended. These structures, (*c*), as will be pointed out later, mark the synaptic points on one of the pairs of V-shaped chromosomes.

The question has naturally arisen as to the behavior of these V-shaped chromosomes in synapsis. Does the process of conjugation begin at the apices of the V's and proceed toward the ends, or does it begin at the free ends and proceed toward the apices? The drawings shown are not entirely conclusive on this point, though very suggestive. In figure 2 the two synaptic points are marked by deeply stained knobs to which the 'vesicles' are attached. These knobs are not situated at the proximal side of the nucleus, but appear to have been pulled away from this position which they undoubtedly held in the telophase of the last spermatogonial division (figs. 7 and 8). The knobs are at the apex of an angle made by a sharp bend in the threads at that point, and while the knobs themselves stand somewhat apart, the threads appear to be conjugated for a short distance on either side of them. When I attempted to follow these conjugated threads they soon became lost in the maze of unconjugated, single threads. The appearances, however, indicate that the only parts of these threads which had conjugated at the time were those immediately adjacent to the appendage-bearing knobs, and therefore at the apices of the V's.

In figure 3 a situation is presented somewhat similar to that in figure 2, except that here conjugation has taken place on only one side of the apical knobs, the remainder of this pair of threads apparently consisting of unpaired leptotene strands. In figure 4, conjugation of this pair has gone further, but even in this case the conjugated part is confined to the region on either side of the synaptic points. These cases, together with others which have not been drawn, tend to support the idea that conjugation of the atelomitic chromosomes begins at the apex of the V, which, as the point of spindle fiber attachment, is homologous with the proximal end of the telomitic or rod-shaped chromosomes.

As soon as conjugation is complete, all of the chromosome threads appear double, as shown in figure 5, which represents the pachytene stage. In this nucleus, the usual orientation of the chromatic elements has been lost. There is some tendency for the threads to be centered toward the side of the nucleus which is uppermost in the drawing, but the accessory chromosome (X), which is customarily attached at the proximal pole of the nucleus, is here removed a considerable distance, and separate from the deeply stained loop (5), to which it is usually attached. This deeply-staining thread is found in the later stages to be that of chromosome 5 (see plate 1) and it very commonly lies near, and often attached to, the accessory. This association may be seen in figures 1, 4, and 6.

Figure 6 represents a nucleus at about the same stage as cell A on plate 1 and shows the separate spireme segments. Each of these has but one longitudinal split, but each represents a tetrad. The segments are numbered according to the arrangement on plate 1. A series of successively older stages may therefore be recognized, beginning with figure 1, extending through figure 6 on plate 3, and continuing through cells A to H on plate 1.

The series of stages just mentioned constitute, it seems to me, a sufficient body of evidence to indicate that in Chorthippus parasynapsis is the rule.

d. Chromatid movements. Attention may be directed to the different rates of change in the chromatid relationships experienced by different chromosomes in the same cell and by the same chromosome in different cells. The typical form of the metaphase chromosome of the telomitic type seems, in this species, to be the rod extended parallel to the spindle axis. Such a form is taken by chromosomes 1, 2, 4, and 6 of cell G on plate 1. Although number 5 has not been pulled out in the direction of the mitotic poles sufficiently to make it a rod, it is readily apparent that only a small amount of movement of the oppositely directed pairs of chromatids would produce the forms seen in chromosomes 4 and 6. As an example of the different degrees to which the chromatid movements have brought about an approach to the extended-rod condition of the metaphase, attention may be called to cell E, where chromosome 4 seems to be lagging much behind the other chromosomes, though in cells F, G, and H, such is not the case. In cell D, on the other hand, chromosomes 2 and 5 seem to be farther advanced toward the metaphase-rod condition than any of the others.

When we examine a particular chromosome as it appears in different cells we do not always see a consistent progress in development in the successively later stages. Chromosome 4 in cell E seems to be in about the same condition, so far as chromatid movement is concerned, as it is in cell C; that is, either one could, by the simple rotation of the free arms, assume the condition seen in cell G. However, it is just possible that in cell D a valid intermediate stage is represented and that this chromosome in the condition found in cell C would have to pass through a stage such as that in cell D before reaching the condition seen in F or G.

e. The planes of the first division. In the absence of well-marked polar granules it is impossible to determine which are the proximal, or synaptic, ends of the chromatids, but if we may assume for the time being that the ends at the left in cells B to E are the synaptic ends, we may trace for chromosome 4 successive steps as follows: In cell B the longitudinal split on the right (the only one visible for two-thirds the length of the tetrad) is probably the primary longitudinal split. The split just forming at the left and continued to the point *a* is probably the secondary split. In cell C, then, we may consider that the formation of the secondary split has been completed and that the chromatids have separated along this split almost to the distal end. Such an element might remain in this condition, condensing through a stage such as is shown in cell E and finally opening out to form an element in the metaphase like the one in cell G. In cell D we may have a stage in which the secondary split has become complete but the separation along its plane has not. If the progress of separation along this plane were to be impeded by gradual condensation, this stage might be followed by a cross formed by the proximal ends (left) of the two pairs of chromatids becoming extended in opposite directions in the same plane (as shown in chromosome 2, cell D), which cross might reach the metaphase in some such form as shown for chromosome 5 and 6, cell F, or even chromosome 4, same cell. A similar series of stages occurs for chromosome 2 in cells B, C, and D. Series of movements such as have been suggested would correspond in all essentials to the series of changes found to occur for chromosome A in Phrynotettix (see '16, fig. 62, plate 6), and would indicate an equational division. Other interpretations, indicating a reductional division are, of course, possible, and cannot be excluded.

In chromosome 6 a similar series of stages may be traced. In cell B the split running through most of the length of the element from the right is probably the primary split, while that just beginning at the left and extending to point *a* would be the secondary split. A continuation of the secondary split and a separation along its plane would give rise to such a stage as that seen in cell C, or, if the separation were delayed, to that in cell D. A rotation of the arms of the tetrad in cell C would give rise to a stage such as is shown in cells E and G, while a similar rotation of the arms of the example in cell D would give rise to the condition seen in F.

It is regrettable that the polar granules, which do occur to some extent, are not sufficiently differentiated by the staining method used to make the orientation of the tetrads certain. In sectioned material stained with iron-haematoxylin polar granules are, in fact, better differentiated than in the safranin stained smear, but in this instance little study has been made of sections, where identification of all the chromosomes is not so certain.

In the case of the pairs of atelomitic chromosomes (nos. 7, 8, and 9) variations in rate and extent of chromatid movement are not so apparent in the examples drawn on plate 1. Such variations do exist, however, as will be seen by comparing number 8 with three 'rings' in cell C, and two 'rings' in cells D, E, and F, and figures 9 to 14, plate 3.

f. The apical constrictions and the chromomere-vesicles. In these atelomitic chromosomes the synaptic point is in many cases very clearly marked by a constriction, as Robertson ('16), has emphasized. This constriction (point *b* in the figures, plate 1) is not visible in all cases, as is indicated in the figures. Chromosome 9 in cell E for example, shows scarcely any evidence of a constriction, although it is plain enough in chromosomes 7 and 8. In cell F chromosomes 7 and 9 show the constriction, while it is not apparent in chromosome 8. Again in cell G chromosome 9 did not reveal the constriction as did chromosomes 7 and 8. All three show evidences of a constriction in cell C.

In connection with this synaptic point, as already mentioned, there is a small plasmosome-like appendage, or chromomere-vesicle (marked *c* in the figures), which seems, in the earlier stages at least, to be as constant in its occurrence as is the constriction just described. It disappears in the later tetrad stages as such structures usually do. Similar appendages are occasionally seen attached to the synaptic points of chromosomes 7 and 8, as is indicated for the former in cell D and for the latter in cell A, but their occurrence on these chromosomes is rather infrequent.

It will be noticed that only one appendage is to be seen on a single chromosome in the figures on plate 1. In earlier stages, however, there are two such bodies as is indicated in figures 2, 3, 4, 6 7, 8, and 9, plate 3. Figure 5 shows the two partially fused together and many times they appear to be entirely fused at these stages. It could not be determined whether the single appendage in plate 1 and in figures 10 to 14 on plate 3 represent two fused together, or whether one of them disappears before the other. The latter possibility is suggested by a constant difference in size in all cases where two are in evidence and by the fact that the single ones in figures 10 and 11 do not appear to be equal in bulk to two such as are shown in figure 9. In figures 12, 13, and 14, they appear larger, but these figures are taken from sections from another individual, which fact may account for their larger size. That these structures are constant elements of this pair of atelomitic chromosomes is well illustrated by the spermatogonial telophases shown in figures 7 and 8. In each case the 'vesicle' is attached at the apex of a V-shaped chromosome with a marked disproportion between the lengths of the arms, comparable to that seen for chromosome 9 on plate 1. In figure 8, the smearing process has resulted in a slight distortion of one of the atelomitic chromosomes (at the left) to which an appendage is attached. It seems fairly safe to regard these chromomere-vesicles as diagnostic for the apices in this largest pair of V-shaped chromosomes.

In the figures from sections (figs. 12 to 14, plate 3) the appendage is attached to only one member of the pair of knobs which mark the synaptic point, and, further, the attachment is always to the knob nearer the middle of the chromatid. In the smears this position is not so evident owing to the lack of definite knobs showing at the synaptic points. However, chromosome 9, cell A, does show such knobs and it will be observed that the appendage is attached to the one nearer the middle of the chromatid. Chromosome 8, cell A also shows such a relationship. The possible evidence that these structures furnish toward an understanding of the planes of first spermatocyte division will be considered in the discussion.

g. The accessory chromosome. The accessory chromosome does not require description or discussion in this paper, but attention may be called to the vesicular appendage attached to it. This appendage is similar to those just mentioned which are attached to the atelomitic chromosomes. This structure seems to have been overlooked by all those who have studied this form, except Davis ('08).

h. Summary. In summarizing the observations on Chorthippus we may call attention to the following:

1. The number of chromosomes in the reduced series (first spermatocyte) is nine, consisting of one unpaired accessory chromosome and eight pairs of autosomes or euchromosomes. Of the latter there are three pairs of atelomitic and five pairs of telomitic chromosomes.

2. The leptotene threads conjugate two-by-two throughout their length during the zygotene, or lepto-zygotene, stages. They remain conjugated through the pachytene stage and until the tetrads are formed. This condition of parasynapsis holds for all the euchromsomes, both telomitic and atelomitic.

3. The process of parallel conjugation (parasynapsis) appears to be inaugurated at the proximal ends of the leptotene threads as was the case in Phrynotettix, and it appears to begin at the apices of the atelomitic chromosomes.

4. A constriction occurs at the synaptic points in most of the tetrads of the atelomitic chromosomes.

5. A small plasmosome-like appendage, or chromomere-vesicle, is attached to the conjugants of the largest pair of atelomitic chromosomes (no. 9) at the apex of the V (synaptic point. A similar appendage is occasionally found at the homologous point on the other two atelomitic pairs, and occurs very constantly on the accessory chromosome.

B. *Trimerotropis suffusa*

a. Introductory statement. Trimerotropis belongs to the subfamily Oedipodinae of the family Acrididae. A discussion of some systematic problems connected with this genus will be found in the paper by Dr. Carothers ('17). The material from which the drawings were made consisted of a single smear made by Dr. Carothers and loaned to the writer for study. This individual corresponds to her number 34 (plate 6) ('17) and cell E on my plate 2 is the same as that drawn by her. Other individuals were studied but no drawings from them are used in the plates.

The reasons for using a smear preparation in this case are the same as those already stated in connection with Chorthippus. This smear was fixed in Flemming's solution and stained by Flemming's tri-color method. The chromatin was not so deeply stained in this smear as it was in the smears of Chorthippus, and this difference appears in the drawings.

b. The post-spireme chromosomes. As Carothers has found, the reduced number of chromosomes in this genus is twelve. Occasionally, however, supernumerary chromosomes occur, as she discovered, and one such supernumerary occurs in this individual, making the number of first spermatocyte elements thirteen instead of twelve.

The arrangement of the chromosomes in plate 2 is the same as for Chorthippus in plate 1. Each vertical column represents the complete series of chromosomes from a single cell arranged in the order of size with the smallest at the bottom.

The differentiation on the basis of size is not always easy to make. This difficulty is made more apparent by the differences between the order used on this plate, where the accessory is made number 3, and the order used by Carothers, who made the accessory number 4. Such differences, however, are without significance so long as other means of identification are available to obviate confusion. The supernumerary is placed at the top of the plate to avoid any possibility of its becoming confused with the smaller tetrads. From left to right are shown successive stages in the transformation of the spireme segments to tetrads, and their condensation, final orientation, and partial division in the spindle of the first maturation mitosis.

A glance at the metaphase figures shows that the complex of this individual consists of four pairs of atelomitic chromosomes (nos. 7, 10, 11, 12), five pairs of telomitic ones (nos. 2, 4, 5, 6, 9), two heteromorphic pairs (nos. 1 and 8), the atelomitic accessory (no. 3), and the supernumerary. In other individuals of the species the relative numbers of pairs of atelomitic, telomitic, and heteromorphic pairs may be different, as found by Carothers and described by her.

It is not possible in every case always to identify each chromosome in a cell. This is due to similarities of size and organization. Chromosomes 11 and 12, for example, appear very similar in size and organization in the earlier stages, so that it is not always possible to tell which is which. In the metaphase and anaphase, however, these two are distinguishable by the different proportions between arm-lengths of the V's. Number 12 has the greatest disproportion between arm lengths; in number 11 the disproportion is less, and in number 10 still less. The proportions between arm-lengths for these atelomitic chromosomes can the more readily be determined by reason of the occurrence in many of the metaphase examples of constrictions or transverse clefts at the points of spindle-fiber attachment. Such clefts are to be seen, for example, in cells E, F, and G for chromosomes number 11 and 12, and cells E and F for number 10. Such clefts or constrictions (marked *b* in the figures) can sometimes be distinguished in the earlier stages as, for example, one cleft for number 12 in cell D, two clefts for number 11 in cell D, two clefts for number 10 in cell C, and in the case of the atelomitic member of number 8 the cleft shows very plainly in cell D. Clefts are likewise indicated for number 7 in cells C and D, and still more plainly in the metaphase cells, E and G. These clefts or constrictions appear to correspond in all respects to those found in the atelomitic chromosomes of Chorthippus.

Chromosomes 5, 6, and 7 are somewhat similar in organization, each possessing polar granules at both ends. This gives rise to some uncertainty as to identity, especially in cells B and C. In cell A the differences in length are more apparent.

Number 4 in cells A, B, and C, and number 8 in cells A and B are readily identified by the peculiar clusters of granules (c) attached to them. In the later stages these disappear and this gives rise to some difficulty in identification in the case of chromosome 8. Number 4, however, possesses an additional means of identification by reason of its staining power which is constantly greater than in the other euchromosomes.

In spite of the difficulties of identification, it is felt that with all the chromosomes present in each cell, and with a careful consideration of the size and structure relations of the various chromosomes, very few mistakes in serial arrangement have been made.

c. The evidence on synapsis. The evidence for parasynapsis, as presented on plate 2 is of the same nature as that for Chorthippus, and consists in the facts: (1) that all the euchromosome pairs, irrespective of the point of spindle-fiber attachment, come out of the growth period as longitudinally split, attenuated threads, and (2) that from these split threads tetrads arise by the formation of the secondary longitudinal split. Figure 21, plate 3, gives the evidence for parasynapsis as it occurs in the lepto-zygotene stages and the conditions shown are quite similar to those already described for Chorthippus and shown in figures 1 to 4. Figure 21 does not represent nearly all of the chromatin threads in the nucleus and is partially diagrammatic. However, the condition indicated—namely, the presence of double threads with one end at the proximal pole of the nucleus and the other end separating distally into two single threads—was clearly seen for a number of those represented and indicated for the others. The deeply staining body near the accessory (X) is regarded as the supernumerary chromosome. In this stage in this material none of the atelomitic chromosomes could be identified and, while the tendency for conjugation to begin at the proximal ends of the threads is clearly indicated, the behavior of the atelomitic chromosomes could not be followed.

d. Chromatid movements. Differences in rates of development, or progress toward separation at anaphase, in different cells find some striking examples in the series drawn on plate 2. Chromosome number 1 seems to be lagging somewhat behind the others in cells B and C, though in cell D it is as far advanced as any. In cell D, number 2 is not so far along toward its typical metaphase condition as it is in cell C. The same relations appear to hold for chromosome 5 in these two cells, yet all of the chromosomes in cell D are more condensed and therefore presumably nearer metaphase than they are in cell C.

In the metaphase there are also some striking differences. Number 4 in cell G is very far behind the other members of the complex and seemingly little advanced in chromatid movement beyond the condition seen in cell A. In cell F, too, separation is not so far advanced for this chromosome as it is in cell E. Cell H is in early anaphase, and cells E, F, and G are at about the same metaphase stage, yet chromosome 9 is not so far advanced toward complete separation in F as it is in E, and in E it is not so far along as in G, yet in G it seems further along than in H. Again number 6 is lagging in cells F and G, as compared to the conditions in E and H. The examples of this chromosome in cells F and G are at about the same stage, but in G a ring exists through the contact of the ends of the arms at the right which are seen to be free in cell F.

All these differences seem to indicate that the extent of chromatid movement is variable, not only for different chromosomes but also for the same chromosome in different cells, so that in some cases the movement which ends in the separation accomplished in the anaphase may be well advanced before the element reaches the metaphase, while in other cases these movements are largely deferred until after the element has been oriented in the metaphase spindle.

There are some pronounced differences in shape of the atelomitic chromosomes in the metaphase. In the case of chromosome 12, for example, a different shape is seen in each of the cells, E, F, G, and H. It is easy to see how a simple movement of the oppositely directed dyads in cell E toward their respective poles would give rise to the condition in cell H; but no such simple movement would account for the shapes in F and G. The explanation of these various shapes, I think, may lie in the statement made above as to the variations in chromatid movement in the earlier stages. A search among the tetrad figures of these earlier stages should reveal the forerunners of the shapes seen in metaphase. In cell C, for example, is a condition of chromosome 12 which would readily give rise to that in cell E if the shorter arms of the V's are toward the right and the apices of the V's are pulled out toward opposite poles. Similarly for chromosome 11, the tetrad in cell C might easily take its place in the spindle in the form shown in cell E and the tetrad of number 10 in cell D could, by orientation, assume the shape in cell E. In cell D, on the other hand, occurs a condition of number 12 which in many respects is similar to that in cell F. The chief difference is that in the latter the short arms of the V's have been reflexed toward the longer arms. The tendency for the shorter arms to bend back in this way when they are free is not infrequent among the atelomitic chromosomes, as shown by McClung ('14). A somewhat similar metaphase of number 11 or 12 from another cell is shown in figure 18, plate 3. Figure 17 shows an earlier tetrad which could give rise to it by the separation of the ends on the right and their flexure backwards toward the left.

None of the earlier stages for chromosome 11 or 12 as seen on plate 2 indicate a condition which could by any simple movement give rise to the shapes seen in cell G. Here the curious condition is found of the shorter arms remaining in contact, while the longer arms have become free. From the standpoint of simple mechanics it would be difficult to understand how a tetrad such as is seen in cell C, for example, could give rise to the condition in cell G. It would be natural to suppose that the crossed longer arms (at the left) would require a greater amount of force for separation than the shorter arms in contact at the right; the pull of the spindle fibers would be expected to be exerted more strongly on the shorter ends. The conditions in cell G are the exception rather than the rule, and I have not found an earlier tetrad which showed the longer arms free as they are in this cell. However, in figure 19, plate 3, there is a ring-shaped tetrad which, by the mere separation of the lightly attached ends of the long arms at the left, would provide a condition analogous to that in figure 20, which resembles those in cell G, plate 2. A condition which is the reverse of that in figure 20 is shown in figure 16, where the short arms are free. Such a shape could readily arise from that in figure 15 by a little more separation along the plane between the arms at the right and their flexure at the point of spindle-fiber attachment.

It might be remarked that one cannot be certain whether chromosome 11 or 12 is represented in figures 15 and 17 because the synaptic points are not indicated by clefts as they are in figure 19. Figure 16 is doubtless number 12, with its great disproportion between arm lengths, while figure 20 is more probably number 11. Figure 18 is problematical.

In cells E and G chromosome 7 (plate 2) seems to have the shorter arms united and the longer arms free, while in F the longer arms are attached to each other and in H they have been the last to separate. In cell E the difference between arm lengths seems much less than in the other cells. This is to be accounted for by the foreshortening of the longer (free) arms and by the stretched conditions of the attached shorter arms, shown by their smaller diameter.

As a net result of such observations one may conclude that the movements of the constituent chromatids of a tetrad are subject to great variation and that with respect to the separation of the ends, there is sufficient evidence to show that either the longer or the shorter ends may become free in advance of the other end. Just why the chromatids should assume the varying relationships to each other in similar stages of the tetrad transformation period is of course not easily determinable, but the metaphase figures seem to be derived from similar ones in the earlier stages without much movement of the chromatids during the later condensed prophases, nor until the forces in metaphase and anaphase are at work. One may suppose that the movement of the chromatids takes place at different rates in different cells and that, as condensation in the later prophases progresses, such movements are retarded; the result being that whatever stage in transformation has been reached in a late prophase is the one in which the element enters the spindle. The further movements then must be the result of the forces in operation during metaphase and anaphase.

e. The planes of the first division. In Chorthippus, as described on pages 480 and 481 the evidence seemed to favor an equational division for chromosomes 2, 4, and 6 (plate 1) which are telomitic. Evidence of equational division among the telomitic chromosomes of Trimerotropis can be found on plate 2. Chromosome 2, for example, as it appears in cell A, has a very characteristic arrangement for the parts of this element. It has very pronounced polar granules at both ends, those at the distal end being the larger. That the larger granules represent the distal end is shown very well in the metaphase figures E, F, G, and H. A further indication that these larger granules are at the distal end is their behavior toward each other. In all of the cells studied which were at stages corresponding to cells A and B, these large granules stood widely apart from each other. The example in cell A shows this especially well. It would hardly be supposed that the proximal ends of a pair, being the first to conjugate, would behave in this manner, while the distal ends might easily fail of complete pairing. Now, since it is possible to determine which end is proximal and which distal, it is also possible, I believe, to trace the entire series of changes into the metaphase. In cell A the primary split is visible at the distal end (at the right in the figure) of this chromosome, but not through the middle. A split is seen in the proximal end (at the left) but it cannot be determined whether this represents primary or secondary split; in cell B, however, both splits are easily seen and it is further observable that the separation at the proximal end (left) is along the plane of the secondary split. The tendency for the proximal granules to diverge with further separation along the secondary split is seen in cell C. In cell D the relation of the chromatids is much the same as in cell B but they are more condensed. In cells E, F, and H, as I have already noted, smaller knobs or enlargements occur at the proximal ends, while a bulging at the middle of the oriented tetrad indicates the larger distal granules. It seems fairly safe, then, to assume that a tetrad in the condition seen in cell C would become oriented in the metaphase without further change in the relationships of the chromatids, except their further separation along the plane of the secondary split. There seems to be good reason for concluding, therefore, that division is equational for this chromosome.

There is also some evidence that chromosome number 4 divides equationally in this first division. In the metaphase cell E the horizontal components of the chromatids on the right side of the tetrad have stained differently from those on the left. They failed to retain the stain so well and appeared finely granular, as indicated in the drawing, in contrast to the compact, deeply staining chromatids on the left. It is reasonable to suppose that those two chromatids which show this peculiarity belong to the same parent chromosome, and, therefore, that halves of chromosomes are being separated in this division, constituting an equational division. Attention may be directed to the cluster of granules attached to this chromosome. In the examples in cells A, B, and C, plate 2, it cannot be determined just what relation this appendage bears to the chromatids. In figures 22 and 23, plate 3, however, it is plainly indicated that the structure is attached to a chromomere of only one of the conjugated threads. Presumably this condition holds true for all stages. If that be true, then these appendages may be at the distal end, if it be permitted to associate the peculiar staining condition of the example in cell E with the presence of this appendage in the earlier stages.

In the cases of chromosomes 5, 6, and 9, there is no method of distinguishing distal from proximal end in the earlier stages and therefore no deductions can be drawn as to which plane of separation prevails in metaphase.

For the heteromorphic pairs of chromosomes (nos. 1 and 8) there seems to be little doubt that the first division is a segregating one, because of the striking difference in shape of the segregating parts. In this respect they are analogous to the unequal pairs found in Phrynotettix and other forms which divide reductionally in the first division.

f. Additional observations on chromosome organization. Some additional points of interest on the subject of chromosome organization may be indicated. In the first place outstanding chromomeres are much more abundant than in Chorthippus, and this is particularly true of those occurring at the ends of the chromosomes. These polar granules (chromomeres) have already been noted in chromosomes 5, 6, and 7, but they are also prominent in the earlier stages, at least, in numbers 2, 4, 8, and 9. The terminal chromomères are less prominent but easily distinguishable in number 10, still less so in numbers 11 and 12. In most cases, with the gradual condensation of the entire chromosome, these prominent chromomeres become difficult to detect in the later stages (cell D, *e. g.*). In the case of number 2, however, the polar granules are relatively very large and they continue to be recognizable even in the metaphase (cell E).

Secondly, the presence of the appended bunches of granules (*c*) on chromosomes 4 and 8, already noted, may be considered further. As characters for identification of these chromosomes they have been mentioned on a previous page. An examination of the pachytene stages shows these appendant structures to be prominent. Figures 22 and 23 (plate 3) show them (c) attached to their respective spireme segments. Chromosome 4 is further identified in these figures by its greater capacity for holding stain. In both of these figures the appendages appear to be attached to only one of the conjugants of each pair of threads, and furthermore, they appear to be joined to single chromomeres in three of the four illustrations. In both cells, that end of the chromosome, near which the appendages are attached, is united with the end of another chromosome, the opposite ends being free in each case. In figure 23 the burdened ends of these two chromosomes are joined together, the appendages are in contact and, so far as could be determined, may possibly be fused at the point of contact. It will be noted that the chromomere to which the appendages are attached appears to be in about the same position with reference to the end of the segment in both cells.

A study of still earlier stages to determine whether these appendages may be double, that is, one for each conjugant, was not made. In the stages shown on plate 2 the bunches of granules were so disposed as to make it impossible to determine whether or not their attachments were restricted to one pair of chromatids. Structures which were interpreted as representing these appendages were seen in the nucleus from which figure 21 was made but were not drawn because to have done so would have confused the other features which were to be emphasized.

A third point on the subject of organization is the occurrence of the transverse clefts or constrictions at the points of spindle-fiber attachment in the atelomitic chromosomes. These clefts have been described in another connection so that it will be unnecessary to repeat a description of them.

In passing, it may be noted that the distribution of the accessory chromosome with reference to the members of the heteromorphic pairs follows the law of chance, as discovered by Carothers ('17). (See chromosomes 1, 3, and 8, plate 2.)

g. Summary. In this individual of Trimerotropis the following conditions have been found:

1. The reduced number in the first spermatocyte is twelve (plus one supernumerary). The complex consists of five pairs of telomitic chromosomes, four pairs of atelomitic chromosomes, two heteromorphic pairs, and the atelomitic accessory besides the supernumerary. These facts were all previously

determined by Carothers, and the present observations corroborate those made by her.

2. Synapsis consists in a side-by-side union of leptotene threads, which parallel association persists until the breaking up of the spireme into its constituent segments and the production of tetrads by the formation of the secondary longitudinal split.

3. The process of parallel conjugation appears to begin at the proximal ends of the leptotene threads, as was found in Chorthippus.

4. The great variety of metaphase shapes for the same tetrad appears to be correlated with variations in the extent and character of the movements of the chromatids with reference to each other in the earlier tetrad stages.

5. At the point of spindle fiber attachment (synaptic point) in the pairs of atelomitic chromosomes there occurs a transverse constriction, or cleft, similar to those found for the atelomitic chromosomes of Chorthippus.

6. Chromosomes 4 and 8 (plate 2) each bears near one end a cluster of granules which is thought to be homologous with the chromomere-vesicles seen in Chorthippus and other grasshoppers.

III. DISCUSSION

On the basis of the foregoing observations it seems worth while to discuss briefly the following topics: (A) Synapsis, (B) pre-reduction vs. post-reduction, (C) chromosome organization.

A. Synapsis

In considering the subject of synapsis it may be well first to summarize the evidence gained from the study of Chorthippus and Trimerotropis, together with that previously recorded for Phrynotettix, and then to compare these evidences with the results recorded by others who have studied Chorthippus (Stenobothrus). An extended discussion of the literature on synapsis would be superfluous in view of the consideration given the subject by McClung ('14), Robertson ('16) and the writer ('16).

The evidence for parasynapsis as it has been presented for Chorthippus and Trimerotropis on the previous pages is very much of the same nature as that presented in the paper on Phrynotettix ('16). The chief differences are as follows: In Phrynotettix three particular first spermatocyte chromosomes were selected for intensive study because they possessed characters which made their identification possible at most of the stages concerned. For these three chromosomes stages in the transformation of the double-thread spireme segment to the tetrad in metaphase or anaphase were traced. In the present study similar stages have been traced for all of the chromosomes in both species. In Phrynotettix the leptotene and zygotene stages were carefully studied and one of the selected pairs of chromosomes traced through these stages. In Chorthippus and Trimerotropis, lepto-zygotene stages have been found which demonstrate the phenomenon of parallel conjugation, beginning at the proximal ends of the threads, just as was found for Phrynotettix.

In the latter species all of the chromosomes are of the rod-shaped or telomitic type, while in Chorthippus there are both telomitic and atelomitic types and in Trimerotropis there are, in addition to the two types named, heteromorphic pairs with one member telomitic and the other atelomitic. I think the evidence presented shows very conclusively that parasynapsis occurs for all the chromosomes of whatever type in these three species.

When we consider the papers of others who have studied the male germ cells of various species of Chorthippus (Stenobothrus) we find that not all have expressed an opinion on the subject of synapsis, and among those who have considered the subject, there are some differences of opinion.

The work of Carnoy ('85), who studied the male germ cells of Stenobothrus along with those of a large number of other Arthropods, was of a pioneer kind and did not concern itself with the subject of synapsis.

Davis ('08) was the first among those who have studied Stenobothrus to consider seriously the subject of synapsis, and on account of a misinterpretation of the looped condition of the pachytene threads he decided that synapsis consisted in an end-to-end union. He regarded the longitudinal cleft of these threads as having arisen by a splitting of the individual chromomeres of the single leptotene threads, instead of arising from a pairing of chromomeres, as I have shown. He failed to detect the formation of the secondary split, confusing it with the primary, and also failed to notice the zygotene stages with their partially conjugated pairs of threads.

Gerard ('09), on the other hand, did notice the zygotene stage with the partially paired threads and correctly interpreted them as evidence of parallel conjugation. He regarded one of the conjugating threads as more prominent "principale" than the other "secondaire" but I have failed to find evidence for such a distinction. Gerard also noticed that pairing began at the proximal pole and progressed distally, as I have described, and further that orientation was not complete nor entirely persistent in the pachytene stages.

Meek ('11), in his first paper on the spermatogenesis of Stenobothrus viridulus, does not commit himself on the subject of synapsis further than to point out the reduction in number of chromosomes in the first spermatocyte and then to say (p. 12): "This reduction must be effected before the breaking up of the spireme, for I have found no evidence of lateral association of filaments after this has occurred," and then (p. 15): "Probably . . . . a numerical reduction takes place as a result of lateral association of chromatin granules or masses on the reticulum of threads prior to the spermatocyte prophase of mitosis." In a later paper ('12) he concludes that conjugation in pairs actually does take place, though he offers no proof for the statement and it is apparent that he has not observed the critical stages.

McClung ('14) in considering a Stenobothrus-like form along with numerous other species of Acrididae admits the possibility of parasynapsis, but did not make a study of the earlier stages where conclusive evidence is to be found.

Robertson ('16) considers the subject of synapsis in Chorthippus at some length. • His figures 163 to 180 represent about the same stages as those shown on plate 1 of the present paper. The evidence in the two sets of drawings is of the same general nature and consists in the facts that each of the early post-spireme segments is a slender filament with one longitudinal split and that a second longitudinal split appears in a plane at right angles to that of the first, giving rise to the tetrads. So far as this evidence goes, therefore, my work, though done independently serves only to corroborate his.

In regard to the actual process of conjugation Robertson has made no observations, but assumes, in accordance with the work of the Schreiners ('06) on Tomopteris and Salamandra and the work of Janssens ('05) on Batrachoseps, that, in the V-shaped chromosomes at least, the process of pairing begins at the distal ends and proceeds toward the apices of the V's. He is further led to this conclusion by finding interlocking pairs, the peculiar relations of which could readily be explained on such an assumption. He then carries the analogy over to the rod-shaped chromosomes and considers that they probably also begin pairing at the distal end.

My observations, as well as those of Gerard, show that, in a general way, the reverse process takes place, namely, that conjugation is initiated at the proximal end and proceeds distally. In the case of the atelomitic chromosomes the evidence is not quite so conclusive, but that shown in figures 2, 3, and 4, plate 3, indicate that here conjugation begins at the apices of the V's, and proceeds distally. However, I am inclined to believe that the progress of pairing may not always be uniform along the whole length of the threads, but that chance association of the distal ends of a pair would result in their union, even if the region between the ends and the apices had not completely united. Such a condition is shown very clearly in the case of chromosome A in Phrynotettix (see '16, figs. 73, plate 6, and figs. 75 to 78, plate 7), and the interlocking of chromosomes such as that shown by Robertson in his figures 163 and 177 could be explained by such behavior.

In conclusion it may be said that parasynapsis has been amply demonstrated for Chorthippus through the work of Gerard, Robertson, and myself. The evidence is just as conclusive for Phrynotettix and Trimerotropis as found by me, while Robertson found clear evidence in Syrbula. Besides these examples from among the Acrididae, parasynapsis was also found among Orthoptera by Otte ('07) for Locusta, by Morse ('09) for blattids, by Vejdovsky ('11–12) for locustids, by Stevens ('12) for Ceuthophilus, and by Robertson ('15) for Tettigidae.

B. Pre-reduction vs. post-reduction

The old controversy over pre-reduction and post-reduction which has agitated the minds of cytologists for so long a time seems still far from a settlement. Even without considering the subject of synapsis there has been little tendency to agreement and with the assurance that parasynapsis is the rule in a large number of animals (Wenrich, '16) the uncertainty becomes more acute. There is one thing, however, about which I believe we can be certain, and that is that there is no general rule which is followed by all chromosomes at all times, and a failure to appreciate this fact has had much to do with the present unsettled state of this subject. I have shown for Phrynotettix ('16) how chromosome-pair C (type C_1) divided by pre-reduction half the time and by post-reduction half the time. In the case of chromosomes A and B the rule was post-reduction, and I believed that post-reduction was the rule for the remainder of the euchromosomes. In that paper (quoted on page 472) I was also bold enough to declare my belief that pre-reduction was the rule with the atelomitic chromosomes of Chorthippus. My greater familiarity with the atelomitic chromosomes since my study of Chorthippus and Trimerotropis has somewhat shaken my faith in some of the criteria used to determine this point. I am strongly inclined to believe that the mode of division in the first spermatocyte cannot be determined with absolute certainty in the absence of recognizable differences between the two conjugants of a pair.

In this connection it may be of interest to review the evidence on this subject as found in the three forms that I have studied (Phrynotettix, Chorthippus, and Trimerotropis), and then to compare the opinions of some other investigators, especially those who have studied Chorthippus (Stenobothrus). It will be necessary to consider only the chromosomes of the first spermatocyte, for the behavior in this first division will govern the subsequent behavior in the second spermatocytes.

In the case of the telomitic chromosomes of Phrynotettix, unequal chromosome-pair C, type C, divided half the time reductionally and half the time equationally. Chromosome-pair B, unequal type, always divided equationally while the equal type behaved in precisely the same manner and presumably also divided equationally. Chromosome-pair A divided equationally with the possible exception of the ring-shaped forms, which were not traced to the metaphase and their behavior not determined.

In the case of the telomitic chromosomes of Chorthippus no very conclusive evidence is available, but a probable behavior leading to an equational division has been described on a previous page (480). Among the telomitic chromosomes of Trimerotropis, number 2 (plate 2) exhibited convincing evidence of an equational division, and good evidence for a similar type of division was found for number 4.

When we come to consider the pairs of atelomitic chromosomes, a decision as to pre- or post-reduction is not so easily reached. The evidence in relation to the chromomere-vesicles found in Chorthippus may be made to favor pre-reduction. In plate 1, it will be remembered, whenever a vesicle occurred it was always single and attached to but one pair of chromatids. This single one might be accounted for in one of two ways: either the two that are found in the earlier stages (figs. 2, 3, and 4, plate 3) have fused together to form a single body, or there may be a normal difference between the two causing one to disappear before the other. In all cases where two are seen, one is slightly larger than the other. This is well shown, for example, in figure 9, plate 3, which presents one of the few cases encountered in which both vesicles appeared at so late a stage. Figures 7 and 8 also show this difference. In figures 10 and 11, the vesicle that is present does not appear to be any larger than the largest one in figure 9, and this fact lends support to the idea that the smaller one disappears early instead of fusing with the larger. The cases shown in figures 12, 13, and 14 are from section of another individual and can therefore not be discussed in this connection. If we assume, then, that the pair of chromatids to which vesicles are attached in chromosome 9, cells E and F, and in chromosome 7, cell D (plate 1), for example, represent one of the conjugants, then the evidence seems clear that a reductional division should take place.

On the other hand, if it be assumed that the two vesicles have fused to form one, then one of them must have broken loose from its former attachments when the pairs of chromatids separated, as they do in the tetrads, whether or not the separation is along the primary or the secondary plane. When it is considered that these structures are eventually destined to disappear in the later tetrad stages, as shown by the fact that many of the tetrads observed in the stages represented by cells C to E (plate 1) do not show any vesicles, the assumption made above does not seem unreasonable. But, if these vesicles are to be regarded as shifting in their attachments, then it might be objected that such structures offer uncertain bases for deduction. On this account, deductions made on the basis of the behavior of these vesicles in the later tetrad stages may have to be considered as not entirely satisfactory. Nevertheless, because of the constancy of occurrence of these bodies throughout the pachytene stages, and the constant difference in size between them, as already mentioned, the most reasonable conclusion would appear to be that the one remaining vesicle, as found in plate 1, and figures 10 and 11 on plate 3, is attached to one conjugant and that therefore, a reductional division is foreshadowed.

We may attack this problem of reduction in the atelomitic chromosomes from a different angle. In chromosomes 2, 4, and 6, plate 1, a probable behavior of telomitic chromosomes was described which paralleled that of chromosome A in Phrynotettix, and consisted in a first separation (though sometimes very slight) along the primary split, followed by a progressive separation of a more pronounced nature along the plane of the secondary split. When we attempt to find an analogous behavior in the atelomitic chromosomes we meet with difficulties. If, however, we follow the example of Robertson ('16) and consider each of the atelomitic chromosomes as composed of two telomitic ones joined at their proximal ends and then consider that the behavior of these component rods is analogous to that described above for the telomitic chromosomes, we would have to conclude that division of these pairs of V's was equational.

If we carry this idea of the compound nature of the atelomitic chromosomes over to Trimerotropis, further difficulties await us. Robertson uses the idea of compound chromosomes in Chorthippus to indicate that the real number of chromosomes is 23 (♂) instead of the apparent 17 (♂), and that, consequently, Chorthippus possesses the number typical of the Acrididae. If we assume that the atelomitic chromosomes of Trimerotropis also are compound then we should have 34 as the total number of telomitic chromosomes in this particular individual (see p. 506). However, if we are to consider the number of chromosomes in the Acrididae to be constant or nearly so we must conclude that the atelomitic chromosomes of Trimerotropis are not compound and therefore we cannot carry over the analogy of behavior from the possibly compound chromosomes of Chorthippus.

On the other hand the behavior of the chromatids may be considered as variable, so that at one time separation in any pair of atelomitic chromosomes could be along the primary plane and at another time along the secondary plane. In this connection, consider for a moment figure 11, plate 3. Here, there are three successive 'rings' with a potential fourth at the right—that is, if the two free ends were in contact, as they are at the left. Each succeeding ring is in a plane at right angles to those adjacent. If we may number these rings 1 to 3 beginning at the left and consider the incomplete ring on the right as number 4 it will facilitate discussion. We might consider either plane as primary or secondary but let us assume that number 3 has the chromatids separated along the primary plane, with the vesicle attached to the chromatids of one conjugant. This tetrad is at a stage early enough so that it could be expected to undergo chromatid movements before it became condensed and oriented in the metapase. If ring 3 has arisen from a separation along the primary split, then the free ends (no. 4) must have separated along the secondary split. So, also, number 2; while number 1 would have separated along the primary split. Now the question may be asked if there is any reason why the further separation of chromatids should prevail along one plane rather than along the other. Does not, for instance, the greater extent of the separation in ring 2 and the smaller size of ring 3 indicate that further movement is likely to be in the plane of separation of rings 2 and 4 which we have assumed to be the secondary plane? Would not such a process give us a condition similar to that in figure 10 with the long free arms at the right having opened out beyond the apical point? Again, could not separation proceed to the enlargement of ring 3 at the expense of 2 and 4 and give rise to such a form as occurs in figure 14?

Similar situations are presented in the series from Trimerotropis in figures 15 to 19. In figure 15, so far as can be observed, there would be just as much chance of separation along one plane as the other, since we cannot recognize the synaptic point in this element. In figure 17, the middle ring might become enlarged so as to produce a condition such as is shown in figure 19, or the ends at the right might be separated and reflexed toward the left as indicated in figure 18, as previously mentioned (p. 488). In any case the primary cannot be differentiated from the secondary plane of separation, and in many ways it seems as reasonable to assume one as the other as likely to prevail in anaphase.

From the heteromorphic pairs another line of argument may be developed. If we assume as established that the heteromorphic pairs in Trimerotropis always divide reductionally as they appear to do, we have a basis upon which to argue that the other atelomitic chromosomes should behave in the same manner and thus divide reductionally. As a matter of fact, chromosome 12 in cell D, plate 2, shows a constriction in one pair of chromatids (b) and not at the homologous point in the other pair. This might be considered as evidence that the constricted pair represented one conjugant which showed this peculiarity without its being shown by its partner, and that therefore a reductional division was foreshadowed. The results of Carothers ('17) also go far to support pre-reduction for the pairs of atelomitic chromosomes. She finds that for each of the chromosomes 1, and 4 to 9 inclusive one of three conditions may exist in different individuals: (1) there may be a pair of atelomitic chromosomes, or (2) a heteromorphic pair, or (3) a pair of telomitic chromosomes. Since the members of the heteromorphic pairs separate in the first division, it is reasonable to suppose that in another individual the two members of a pair of atelomitic chromosomes, both of which were homologous to the one atelomitic member of the heteromorphic pair, would also separate in the same way. And further, it might be presumed that even the telomitic homologues, when both are present in the same tetrad, would have a similar behavior. The strongest evidence, then, in both Chorthippus and Trimerotropis seems to support the pre-reduction hypothesis for the atelomitic chromosomes.

I have gone into all these suppositions and analogies for the purpose of showing how difficult it is to arrive at any general conclusion with regard to the behavior of the chromatids and the consequent plane of separation at the first division. It is my belief that only in those cases where the two conjugants of a pair can be differentially recognized can the plane of separation in the first spermatocyte division be absolutely determined for these atelomitic chromosomes.

The method of analogy is perhaps too generally used in the discussions of the subject of reduction. Gregoire ('10) attempted, unsuccessfully, to align most of the results published up to the time of the completion of his paper into an agreement on the side of pre-reduction. Davis ('08), taking the V-shaped chromosomes of Stenobothrus as a type, concluded that pre-reduction occurred in them, and then by analogy, probably occurred in all the others. Gerard ('09), though somewhat uncertain, likewise concludes that the plane of separation of the atelomitic rings is along the primary plane and is therefore reductional and then says (p. 579): "Personne ne contestera cependant que tous les chromosomes doivent se comporter à ce point de vue de la même façon et que l'on est autorisé a appliquer, à tous, ce que l'on a vu d'une façon irréfutable chez quelques uns d'entre eux." I think there is danger in drawing such analogies.

Meek ('11) says he is not able to discover whether reduction occurs at the first maturation division or the next but thinks possibly both divisions are equational and a numerical reduction takes place only as a result of lateral association of chromatin granules or masses on the reticulum of the threads prior to the primary spermatocyte prophase. Meek thus failed to see the parallel conjugation (though he assumes it may occur) and does not recognize the primary longitudinal split (along which one division takes place) as the space between conjugants.

McClung ('14) mentions the difficulty in determining the plane of division in the first spermatocyte and says, p. 665–666: "It is probably true that until the relations of the chromosomes during the synaptic phase are definitely determined it will not be possible to assert unequivocally that the longitudinal axes of the paired chromosomes of the first spermatocyte represents the coincident axes of the spermatogonial chromosomes constituting it, for if there be a parasynapsis at any period there is a possibility that the doubly split thread may open out along the plane of the equational cleavage instead of along the space between chromosomes. In either event the form of the resulting chromosome would be the same." However he believes that (p. 667): "The sister halves of a chromosome remain closely united here as in other generations of cells, and that separation between parts of a tetrad is much more likely to occur along spaces between whole chromosomes." If one were to apply the reasoning in the last sentence quoted to the cases under discussion one would get variable results. Besides, I think I have sufficiently demonstrated parasynapsis and therefore the existence of the basis for the uncertainty mentioned.

Robertson ('16) is persuaded that all the autosome pairs divide reductionally in the first division. He bases this conclusion on the interlocking tetrads which he found in Chorthippus, the behavior of the 'bi-tetrads' in Jamaicana (Woolsey, '15), the segregation of unequal rods in the first division described by Hartman ('13), Carothers ('13), and Robertson ('15), and the pre-reductional division of the multiples in Hesperotettix and Mermiria (McClung, '05).

He says (p. 246): "From the instances which I have here given it seems to me the inference may possibly be drawn that all autosomal tetrads will be found to divide reductionally in the first maturation division."

In Phrynotettix I found one unequal pair which apparently always divided equationally in the first division and another which did so half the time. I was also able to show that Chromosome A in Phrynotettix, though the conjugants were of equal length, nevertheless divided equationally in the first division, and I believe that a similar behavior is clear for chromosome 2 in Trimerotropis. With these different facts established, the variability of behavior in the telomitic chromosomes with regard to reduction must be recognized, and, further, in the case of the atelomitic chromosomes it seems unwise to attempt to establish a general rule.

C. Chromosome organization

a. Atelomitic, or V-shaped chromosomes. The idea of multiple or compound chromosomes was first presented for Orthopteran material by McClung ('05). In a paper in this journal Dr. McClung considers this subject in a comprehensive manner so that it will be unnecessary to discuss here more than a comparison between the chromosomes of Chorthippus and Trimerotropis.

The occurrence of a constriction or transverse cleft at the apices of the atelomitic chromosomes has been noted several times and I have already mentioned the view that Robertson ('16) has taken in regard to the possibly compound nature of the atelomitic chromosomes of Chorthippus. In drawing his conclusion that these chromosomes are compound, Robertson makes two assumptions, namely, (1) that the number of chromosomes for the Acrididae is constant (i.e., 23, diploid series) and (2) that the 'achromatic bridge' or constriction at the apex represents the point of union between two telomitic chromosomes joined at their proximal ends. Were we to generalize on these assumptions and apply them to Trimerotropis a difficulty would immediately arise. In the first place, this form possesses the number of diploid chromosomes typical for the Acrididae (23) in spite of the presence among them of V-shaped members. If we should assume that each of these V's represents two formerly separate rods, then the total number of rods in the individual studied would be 34, as follows: for the 4 pairs of atelomitic chromosomes, 16; for the 2 heteromorphic pairs, 6; for the 5 pairs of telomitic chromosomes, 10; and for the atelomitic accessory, 2. This number (34) would be a radical variation from the supposedly constant number of 23, and in other individuals the number might be more (or less). As shown by Carothers ('17), the range in number of rods on this basis could extend from 30 (individual no. 62) to 40 (individual no. 6). From the standpoint of constancy in number, therefore, the V's in Trimerotropis should not be considered compound.

On the other hand, if it is to be assumed that the 'constriction' is a criterion for the recognition of compound chromosomes, then the majority, at least, of the atelomitic chromosomes in the individual studied would have to be considered compound (plate 2). It is true that the assumption of a compound nature for the atelomitic chromosomes of Chorthippus enables one to recognize the number typical for the group (23), but a similar assumption for Trimerotropis only causes a wide divergence from the typical number which is already present. Perhaps the way out of the difficulty may lie in the suggestion made by Robertson ('16, p. 221) that there are two types of V-shaped chromosomes, one type representing two rods joined at their proximal ends and the other type a bent rod with spindle-fiber attachment in a non-terminal position.

On this supposition the typical Acrididian number may be conserved by assuming that the atelomitic chromosomes of

Chorthippus are of the compound type and that those of Trimerotropis belong to the other type. Considering the similarity of organization of these chromosomes in the two species, i.e., the presence of the apical constriction in the two types, it will be seen that such a constriction cannot be used as a criterion for the recognition of the compound type.

Robertson seeks to extend this compounding idea to the V-shaped chromosomes of other groups of animals. It seems to be unsafe to generalize so extensively, considering that such a generalization does not seem to hold in all cases, even among the Acrididae. It would appear, rather, that each group would have to be considered by itself and conclusions drawn only from a study of numerous species within a group. Such a study has been made by Metz ('14) in which case the compound nature of the V-shaped chromosomes of Drosophila seems to be established. But in this case, as Metz has pointed out, it is as admissible to consider two rods to have arisen from the transverse cleavage of a primitively V-shaped chromosome as to assume that a V-shaped type arose by an end-to-end union of two of the primitively rod-shaped type. As Robertson has noted, Agar ('12) found very decidedly constricted chromosomes in Lepidosiren and that (p. 295): "The constancy of the position at which transverse segmentation takes place indicates a constant differentiation of the chromosomes in a lengthwise direction." But he considered that: "The presence of transverse constrictions or joints in chromosomes is not, without special evidence, to be taken as an indication of bivalency or of a future division plane." This note of caution ought, I think, to be especially emphasized.

b. The chromomere-vesicles. The plasmosome-like chromomere-vesicles were first called 'vesicles' by Carothers ('13). In '16 I called attention to their plasmosome-like appearance, at least in certain stages, and emphasized the point, already noted by Carothers, that they are related to definite regions of chromosomes. Dr. Carothers ('17) now calls them 'chromomere vesicles' (p. 473), a term which emphasizes their relationships to separate chromomeres and distinguishes them from the chromosome vesicles found in the spermatogonia where each vesicle represents an entire chromosome. These structures will be considered here only as further evidences of the exact organization of each chromosome.

As pointed out in the observations, these chromomere-vesicles are attached to the chromomeres at the apices of the V's in Chorthippus—quite regularly in chromosome number 9, less so in numbers 7 and 8—and near one end of the telomitic chromosomes 4 and 8 in Trimerotropis. In Chorthippus one is practically always attached to the accessory.

The relationship of the vesicle to a particular part of this accessory is not readily observable in the growth period, as shown in figures 1 to 6, plate 3, but on plate 1, it is readily seen that the vesicle (c) is attached to a point near the middle but always nearer one end than the other, indicating that in this case, also, the relationship is to some definite region or chromomere of the accessory.

Whenever the chromomeres at the apices of the V's in Chorthippus can be distinguished in the same tetrad which shows a vesicle, it is found that the vesicle is always attached to that one of the pair of chromomeres which belongs to the longer arm of the V. This is seen especially on plate 3 in figure 9 from the smear and in figures 12 to 14 from sections. It is also seen in in chromosomes 8 and 9, cell A, plate 1.

Similarly, in Trimerotropis, the relationship of the granular appendage is constant in the two chromosomes to which they are attached. In plate 2 the relationships are not so definitely ascertainable, because of the more condensed condition of the chromosomes and of the granules in the cluster, but it is noticeable that the cluster is closer to the end in chromosome 8 than it is in chromosome 4. This is better seen in figures 22 and 23 in plate 3. In both cells the clusters are attached to chromosome 8 at or near the last chromomere but one, near the end and in chromosome 4 to the fourth chromomere from the end. In figure 23 the ends of chromosomes 4 and 8 are in contact. The two appendages are also in contact, but it could not be definitely determined whether or not they had fused together. Taken all in all, this brief study of these slightly-understood, plasmosome-like chromomere-vesicles adds considerable to the evidence indicating a constant and definite organization of each chromosome.

D. Summary of conclusions

1. Parasynapsis occurs in all the chromosomes of Chorthippus and Trimerotropis, whether they are pairs of rods, pairs of V's, or heteromorphic pairs.

2. The pairing begins at the proximal end of the rods and at the apices of the V's and proceeds distally. It is probable that the distalward progress is not uniform, but that the distal ends may occasionally become conjugated before intermediate parts have united.

3. In the telomitic chromosomes of Chorthippus and in chromosomes 2 and 4 of Trimerotropis there is evidence that the first maturation division may be an equational one. The heteromorphic pairs probably divide reductionally. In the case of the atelomitic chromosomes the bulk of evidence favors prereduction, but no definite conclusions on this point seems to be possible.

4. There are probably two types of atelomitic chromosomes, one, found in Chorthippus, consists of compound chromosomes, representing two rods joined at their proximal ends, the other, in Trimerotropis, is a bent rod. This is in agreement with Robertson's ('16) suggestion.

5. Chromomere-vesicles are constant in position in the chromosomes to which they are attached, and go to support the idea that the chromosomes are constant in their internal organization.

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EXPLANATION OF PLATES

All figures in the three plates were drawn under a camera lucida at a magnification of 3000 and reduced one-half in reproduction.

PLATE 1

EXPLANATION OF FIGURES

All drawings made from a smear from a single male of Chorthippus (Stenobothrus) curtipennis. Vertical columns represent separate cells, each horizontal row represents one chromosome in different stages. Figures 1 to 9, at the left, refer to the separate chromosomes arranged in order of size. The letters A to H at the top refer to individual cells. *a*, Refers to four strand condition, indicating two longitudinal clefts; *b*, refers to transverse constrictions in the atelomitic chromosomes; *c*, refers to the chromomere-vesicles.

SYNAPSIS AND CHROMOSOME ORGANIZATION PLATE 1

D. H. WENRICH

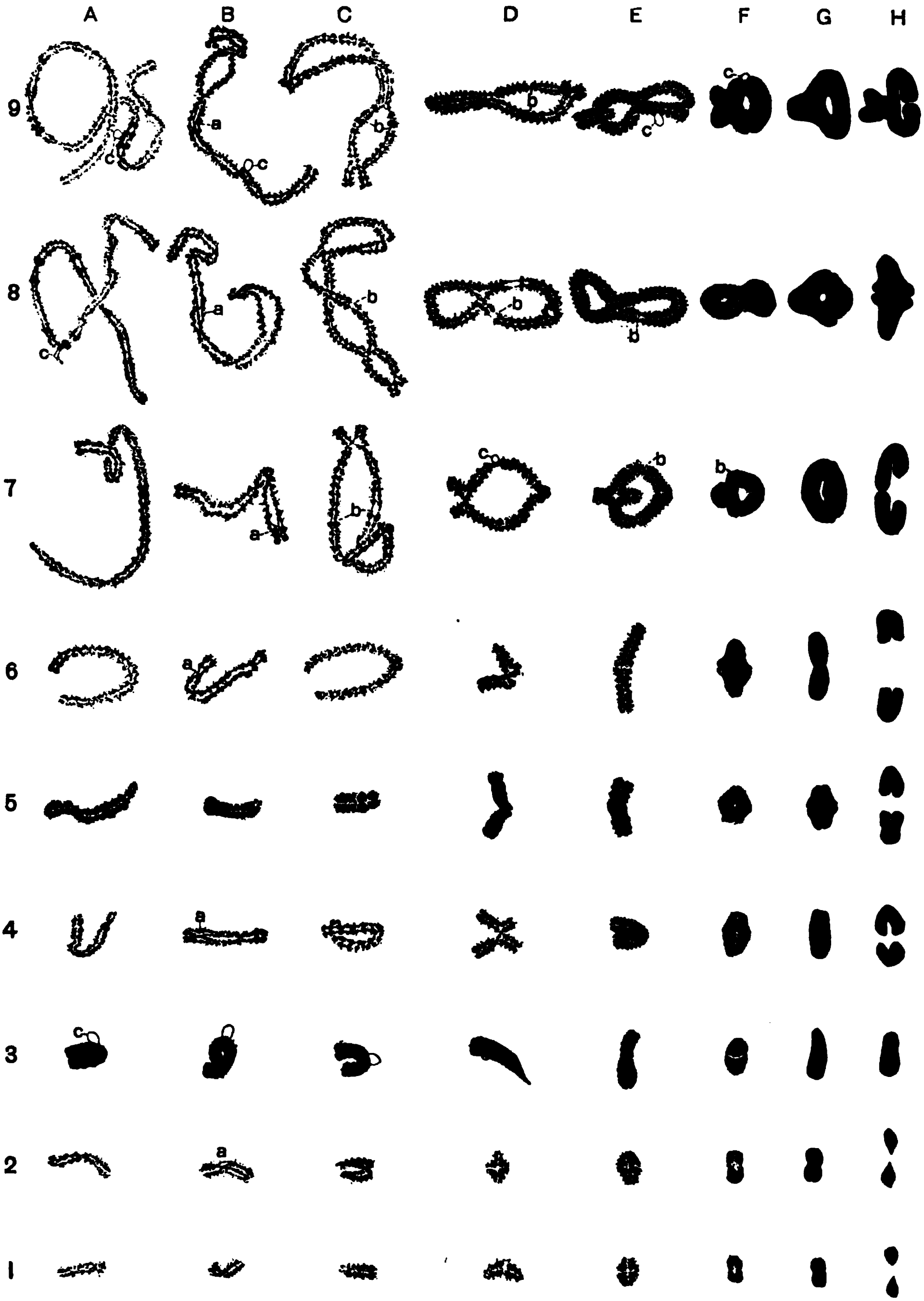


PLATE 2

EXPLANATION OF FIGURES

All drawings from a smear of a single male of Trimerotropis suffusa. Figures 1 to 12, at the left, refer to the different chromosomes arranged in order of size. Letters A to H at the top refer to separate cells. The supernumerary chromosome in the row at the top of the plate is unnumbered. *b*, Refers to the transverse clefts or constrictions in the atelomitic chromosomes; *c*, refers to appendant clusters of granules (chromomere-vesicles) in chromosomes 4 and 8.

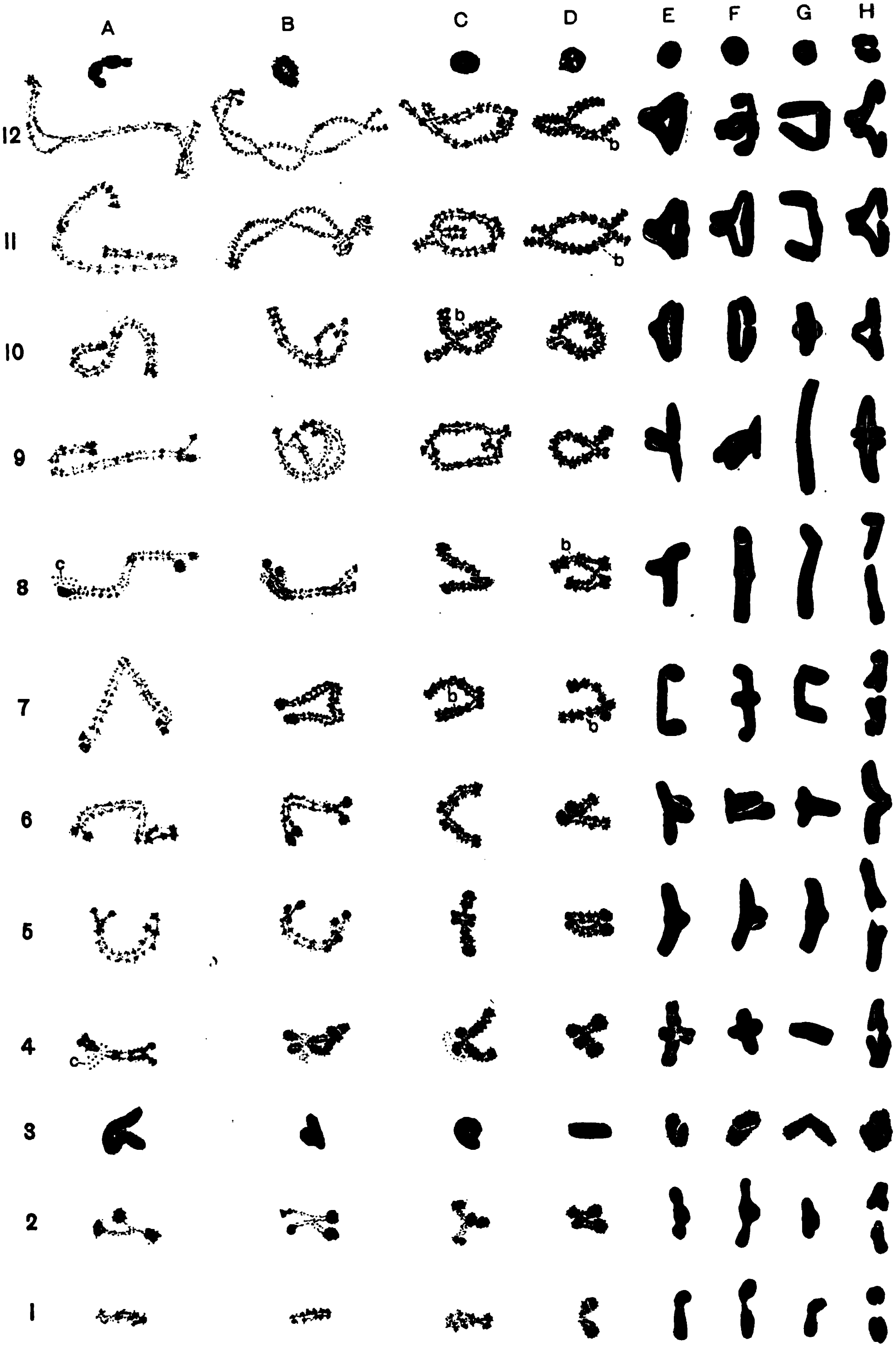

A
B
C
D
E
F
G
H
12
11
10
9
8
7
6
5
4
3
2
1
b
c

PLATE 3

EXPLANATION OF FIGURES

Small numerals in figures 1, 4, 5, and 6 correspond to the numbers of the chromosomes in plate 1. The small numerals in figures 22 and 23 correspond to the numbers of the chromosomes in plate 2. *c*, indicates the chromomere vesicles; *X*, indicates the accessory chromosome.

1 to 11 From the same smear of Chorthippus that was used for the drawings on plate 1. Figures 12 to 14 are from sections of another individual of the same species.

1 to 4 Zygotene stages, showing partially conjugated threads. In figures 2 to 4 the apices of the atelomitic chromosome no. 9 are marked by chromomere-vesicles (*c*).

5 Pachytene stage. Chromomere-vesicles (*c*) partially fused.

7 and 8 Telophases of spermatogonia showing chromomere-vesicles (*c*) attached to chromosomes no. 9. Chromosome vesicle of accessory at *X*.

9 to 11 Tetrads of chromosome 9, illustrating variation in chromatid arrangements at about the same stage.

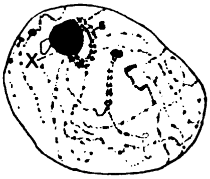
12 to 14 Tetrads of no. 9 from sections showing chromomere-vesicles and variety of chromatid arrangement.

15 to 23 Trimerotropis; from same smear as that used for drawings in plate 2.

15, 17, and 19 Prophase tetrads which may be considered forerunners of the metaphase figures 16, 18, and 20, respectively.

21. Zygotene stage showing partially conjugated threads.

22 and 23 Late pachytene stages showing chromomere-vesicles on chromosomes no. 4, and no. 8.



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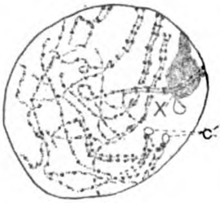

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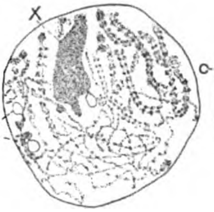

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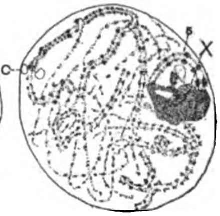

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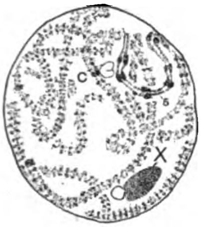

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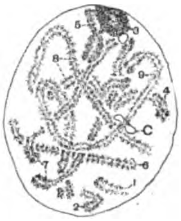

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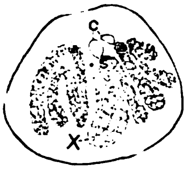

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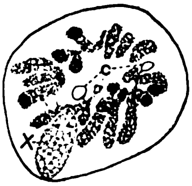

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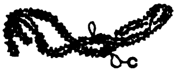

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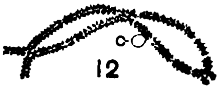

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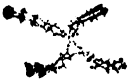

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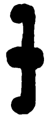

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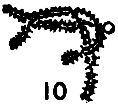

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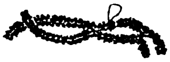

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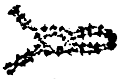

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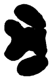

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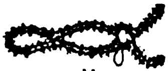

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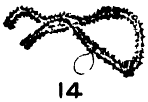

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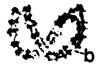

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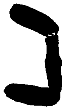

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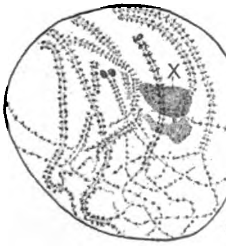

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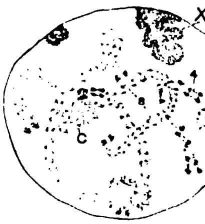

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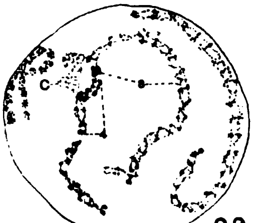

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[*Reprinted from* SCIENCE, *N. S., Vol. XLIII., No. 1118, Page 781, June 2, 1916*]

A NEW COLOR VARIETY OF THE NORWAY RAT

NORWAY rats with dilute coat color have recently been taken in the vicinity of the University of Pennsylvania. If we may judge from the fact that the nine individuals thus far found are all approximately alike and are distinctly different from the normal type, they probably represent a new Mendelian variety.

The coat is intermediate in color between that of the ordinary dark form and the albino and resembles that of the red-eyed guinea pig. In the guinea pig this color has been shown by Wright to be allelomorphic with albinism and with dilute. As in the guinea pig, the hair of the new rat seems to be without yellow pigment and is dilute black or brown ticked with white.

The eyes look black unless the light is very bright. When the light shines directly into them they appear pink. They are distinctly lighter than the eyes of Castle's red-eyed yellow rats, but darker than those of his pink-eyed yellows.

The new rats are now in the care of the Wistar Institute, where the endeavor is being made to increase the stock and to cross with the color varieties already known.

Data in regard to the distribution of the new form is being collected and will later be published.

The previously known Mendelian varieties in the rat are five: black, hooded, albino and Castle's two yellow varieties, red-eye and pink-eye. This new variety is a non-yellow dilute and may be called ruby-eye.

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THE CHROMOSOMES OF THE COMMON HOUSE-MOSQUITO, CULEX PIPIENS L.

BY

P. W. WHITING

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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THE CHROMOSOMES OF THE COMMON HOUSE MOSQUITO, CULEX PIPIENS L.

P. W. WHITING

Zoological Laboratory of the University of Pennsylvania[1]

SEVEN TEXT FIGURES AND SEVEN PLATES

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[1] Contribution from the Zoological Laboratory of the University of Pennsylvania.

I. INTRODUCTION

On account of the contradictory statements published in regard to the cytological conditions of the common house mosquito, Culex pipiens L., it was thought worth while to investigate the matter thoroughly in order to find out the cause of disagreement and to discover the true conditions. This has seemed of especial interest in view of the extensive genetic work that is being done on one of the higher Diptera, Drosophila ampelophila Loew.

While certain other orders of insects have been well investigated cytologically, the published work on the Diptera is very meager. It consists of two papers on mosquitoes by Stevens ('09 and '11), one by the same author on nine species of Myodaria ('08), a paper on Culex pipiens by Lomen ('14), another by Taylor ('14), and a paper on twelve species of Drosophila by Metz ('14). Besides these there are two short reviews, one by Metz ('16), and one by Dehorne ('14), and a genetic paper by Bridges ('16) dealing with non-disjunction of the sex chromosomes in gametogenesis of Drosophila ampelophila and figuring and describing the diploid complexes.

It was thought best to make a thorough study of the spermatogenesis of Culex pipiens and a comparative study of conditions in the ovary and somatic tissue. By reason of improved technique and the encouragement and criticism of Dr. C. E. McClung, Dr. D. H. Wenrich, and Miss E. Eleanor Carothers, I have been enabled to make considerably more out of the material than was at first expected.

A discussion of technique, together with a criticism of the literature, will be reserved until after the descriptive part of the paper. It is believed that the contradictory results obtained by the various investigators have been largely due to differences of technique and it is therefore desirable to discuss these matters after presenting my own results. It may merely be stated here that Flemming's stronger fluid has been used as a fixative except where otherwise mentioned. Culex pipiens may be bred in large numbers throughout the winter months and quantities of material obtained at all times for study.

II. OBSERVATIONS

A. SPERMATOGENESIS

a. Introductory

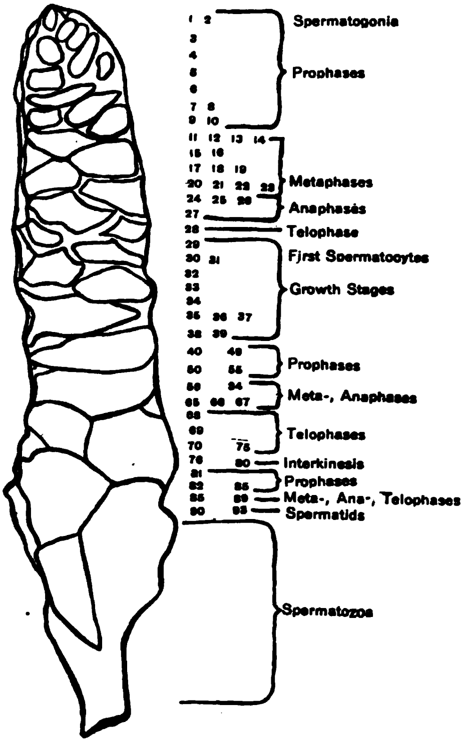
The testes of Culex pipiens are two ellipsoidal, colorless, highly refractive bodies lying laterally in the sixth abdominal segment. Each consists of a single follicle, divided by partitions of connective tissue into a number of cysts. The spermatogonia are formed in the cephalic end and these are followed by the successive stages of spermatogenesis as shown in text figure 1, which is from a longitudinal section of a pupal testis at a magnification of 1800 diameters. The numbers to the right of the figure refer to the successive stages as illustrated by figures 1 to 93. Usually not all stages will be found in a single testis, but I have occasionally found an individual with divisions of spermatogonia, first and second spermatocytes, and with spermatids. The development of the germ cells is not completely correlated with the age of the insect, but considerable variation obtains.

b. Spermatogonia

The last spermatogonial division and the 'resting' nuclei preceding it are the earliest stages found in full grown larvae and pupae. Probably younger larvae would show a greater number of spermatogonial generations.

1. Prophases. The earliest stage obtained is illustrated by figures 1 and 2. The chromosomes are very thin and only partly visible. A large irregular, diffuse nucleolus is a striking characteristic of this stage. If often shows four darker areas (fig. 1). There may be more than one nucleolus (fig. 2), together equivalent, however, in size and staining qualities to the one. The nucleolar elements are clearly attached to the chromatic threads, which may sometimes be traced to the periphery of the nucleus. They show no tendency to be approximated to the side of the nucleus, but occupy various positions in the karyolymph.

Successively later stages, as judged by relative position of the cysts, are shown in figures 3, 4, and 5. The nucleolar elements are darker in these stages. They tend to appear separate at first (fig. 3), and to fuse later. The nucleolus then gradually becomes diffuse while the chromosomes thicken and stain more densely. Figure 6 represents an optical section of a nucleus at this stage. The separate chromosomes can not yet be made out, but a thick chromatic cord is attached to the diffuse nucleolus. Two terminal granules, each connected to the tip of the chromatic thread to which the nucleolus is attached, are of interest as they appear again in first spermatocyte prophases. They are here diffusely stained and suggest by their form the antennae of a butterfly.



Text Figure 1. Location of stages in the gonad

Upon the disappearance of the nucleolus the separate chromosomes become apparent as three pairs of bent threads. The nuclear membrane now dissolves and the chromosomes appear as in figures 7, 8, and 9. Two of the pairs are very much longer than the third, but this difference is not always well seen in the figures on account of foreshortening due to twisting up or down. Figure 9 is a slightly later stage than the others and the chromosomes have shortened and thickened. Figure 10 is a very late prophase.

2. Metaphase, stages a and b.* Figures 11, 12, 13, and 14, represent a very characteristic arrangement of the chromosomes in a flat plate with a somewhat radially symmetrical appearance. This stage may be called stage A of the metaphase. The chromosomes do not begin their division at the end of this stage but first assume a parallel arrangement, which may be called stage B. In stage A the chromosomes tend to be constricted in the middle and swollen at the ends. The homologues are usually approximated and their middles are nearer to each other than their ends. Since the chromosomes are in an approximately flat plate we may speak of a polar or an equatorial view of the same by analogy with the metaphase. Equatorial views are not favorable for drawing, but the chromosomes may be studied and counted by focusing.

In the figures of stage A a short line is drawn to represent the polar axis. The number at one end of the line denotes in degrees of the circumference of a circle the slope of the polar axis to the plane of the drawing. The direction of the polar axis has been estimated by focusing upon the metaphase plate.

The smaller pair of chromosomes is readily distinguishable from the larger pairs in metaphase and anaphase, as it is also in late prophase. There is considerable variation in the size of the chromosomes from different cells, probably depending upon nutritive conditions or previous rapidity of cell divisions.

There are certain points of interest to be noted here in connection with diffuse bodies and extra elements. In figure 9 there is represented an extra darkly stained element alongside one of the big chromosomes in the lower right hand corner. Nothing of the sort can be made out in the cell shown in figure 10. In figure 11 is drawn a diffuse body resembling the nucleolus. Figure 12 also shows a diffuse body as well as a diffuse element attached to the outer member of one of the large pairs of chromosomes. In figure 13 are two diffuse bodies, and in 14 a diffuse end of the inner member of a large pair. This end is much more condensed than that shown in figure 12. In stage B of the metaphase and in anaphase these diffuse bodies and extra elements are not seen.

Figure 15 is a condition frequently observed, in which the chromosomes are passing from stage A to stage B. The members of the small pair are still considerably bent, those of one of the larger pairs appear to be perfectly straight. The radial symmetry of stage A is here lost and the six chromosomes are beginning to assume the parallel arrangement of stage B.

Figure 16 shows a metaphase in which the chromosomes have assumed an approximately parallel arrangement. In the description of this stage and the stages immediately following, it will be necessary to distinguish three axes—the polar axis and two equatorial axes. The polar axis connects the poles of the spindle and passes through the centre of the metaphase plate and at right angles to it. An equatorial axis cutting the chromosomes transversely may be called the transverse axis, while an equatorial axis lying parallel with the chromosomes and passing between the members of the middle pair may be called the parallel axis. In figures 16 to 28 the directions of the three axes are represented by lines, each with a number at one end, which indicates how many degrees of a circumference the end of the line should be raised above the plane of the paper to get the inclination of the axis. The polar axis is represented by a solid line, the transverse axis by a dotted line, and the parallel axis by a dot-and-dash line. Thus figure 16 is a polar view, with the transverse and the parallel axes in the plane of the paper. The three axes are of course always at right angles to each other. If we disregard differences of size and irregular bending of the chromosomes, we see that in stage B the metaphase plate approximates biradial symmetry, the transverse and parallel axes each dividing symmetrical halves.

Figure 17 is the same stage. The shortness of the chromosomes is due in part to sloping of the polar axis, in part to fixation. In figure 18 the shortness of the chromosomes is also due to shrinkage, illustrating the effect of imperfect technique. The figure shows that the small pair may occupy the centre of the metaphase plate. In figure 19, a transverse optical section of the chromosomes in the same stage, the members of each pair are closely approximated, apparently a matter of fixation. Figures 17, 18, and 19 are all from the same testis which showed imperfect fixation. The small pair in figure 19 is on the left, as may be judged by focusing, although the diameter is as great here as that of the others.

Figure 20 shows that the chromosomes begin to split in the middle. The plate is so bent that, although the transverse axis is in the plane of the paper, five of the chromosomes show the beginning of the split. Figure 21 is a similar stage. The chromosome at the right has been pulled out of position by sectioning and broken at the point of attachment of the spindle fibres; the daughter chromosomes are somewhat separated. Figure 22 is a side view of a chromosome from another plate at this same stage and figure 23 is a transverse optical section of a similar plate The ends of the chromosomes seen at the left and right are visible on account of bending. Figure 24 is a side view of a chromosome in a little later stage of division showing part of another chromosome that was in the same optical section. Figure 25 is an oblique view of the chromosomes at a slightly later stage,

3. *Anaphase and telophase.* Figure 26 is an optical section of the ends of the chromosomes just separated in anaphase. Figure 27 shows a later anaphase with the chromosomes still displaying some of their parallel arrangement.

The spermatogonial chromosomes always seem to pull out very thin in anaphase and the members of the homologous pairs are often so close as to be inseparable. In figure 27 the members of the small pair have apparently fused completely.

Bending of the polar axis frequently takes place in anaphase, and in telophase the daughter nuclei may become considerably displaced relative to each other. Figure 28 represents a telophase in which considerable bending of the polar axis has taken place. The directions of the three main axes are therefore given separately for each newly-formed nucleus. The chromosomes still have their arrangement parallel to each other in each daughter nucleus. This is clearly seen at the right but not at the left, as the equatorial axes are sloping.

There is considerable tendency for the chromosomes to become diffuse in anaphase, and in telophase this has gone so far that, with normal staining by the iron-haematoxylin method, the nuclei appear quite devoid of any chromatic material. The slide from which figure 28 was taken was very darkly stained. The telophase nucleus gives rise to that of the first spermatocyte at the division of the cell body.

c. First spermatocytes and nucleolar elements

1. Earlier prophases. The character of the first spermatocyte nucleus shortly after the last spermatogonial telophase is shown in figure 29. The chromosomes are exceedingly tenuous and the nucleolus is very diffuse, with four darker areas. Except for its smaller size the nucleus resembles that of the spermatogonia as shown in figure 1. At a little later stage, as judged by position of cysts, the nucleolus has condensed into two black masses (figs. 30 and 31). Figures 32 *a*, 32 *b*, and 33 show later stages in which the chromosomes are more condensed, nuclear size considerably increased and nucleolus irregular and somewhat diffuse in parts. In figure 34 the nucleolus is very black, but has a light centre. At this stage the nucleolus is always very smooth in outline. Figure 35 is a characteristic condition of a later stage in which the dark area of the nucleolus is surrounded by a lighter granular investment. In figure 36 the diffuse part appears to have been separated from the darker part and forms a pair of terminal granules which are seen very frequently in later stages. In figure 37 the nucleolus has become diffuse, while the chromatic threads are thicker and darker.

The double thread terminating in the granules has become very much condensed near them. It is of interest to see that there are four threads connected with the nucleolus and that at least one of them may be continuous between the nucleolus and the terminal granules. Figure 38 represents a later stage in which the four single threads connected with the nucleolus have fused into two double threads.

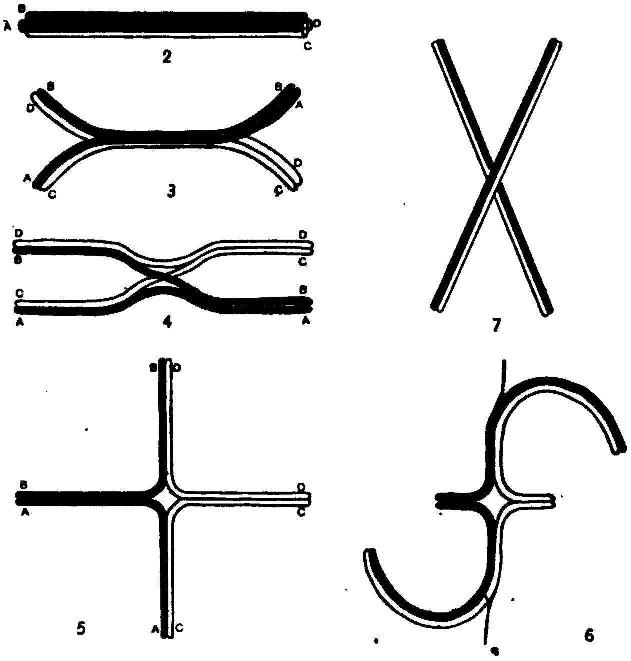
In figure 39 the still diffuse chromosomes appear distinctly as three pairs, the smaller pair at the center of the figure and at a much lower focus than either of the others. The two larger pairs are at the sides with the members united at the ends. The pair at the right shows the terminal granules. The nucleolus is still very black and is at considerable distance from the pair at the right, but connected with the middle of the same by an achromatic fiber.

Figures 40 to 47 are from sections which have been very much overstained in order to bring out the more diffuse elements. In consequence the chromosomes are very black and the members of each of the three pairs appear more or less fused.

Figure 40 shows one of the larger pairs with the diffuse nucleolus attached to its middle and the terminal granules each appearing double. The more distal parts of the latter are very black. In figure 41 the terminal granules are rather large; the attachment of the nucleolus can not be made out. Figures 42 *a* and 42 *b* show a nucleus cut in sectioning. The terminal granules are here very small. The nucleolus, now appearing double, is connected with the large pair of chromosomes which bear the terminal granules. The two other pairs have been broken in the middle, the point of spindle-fiber attachment. Figure 43 shows the diffuse nucleolus and a terminal granule attached to the same large pair. The other terminal granule could not be seen but is probably hidden under the chromosomes. In figure 44 the nucleolus has become exceedingly diffuse and in figures 45, 46, and 47 is not visible. The terminal granules vary greatly in size, in 47 becoming hardly more than faint threads.

2. Later prophases to early anaphases. Tetrads. In order to make clear the conditions shown in the succeeding figures of first spermatocytes, diagrams will be helpful. Text figure 2 represents a tetrad with its four elements, the chromatids. *A* and *B* represent the chromatids from one homologue and *C* and *D* the chromatids from the other.

The formation and existence of tetrads mark the post-spireme stage which ends, according to Wenrich ('16), "with the establishment of the tetrad-chromosomes upon the mitotic spindle of the first spermatocyte division." Since, however, in Culex pipiens, each tetrad passes through late prophase stages, through the metaphase, and up to the anaphase independently of the others, the post-spireme stage must be considered separately for each tetrad.



Text Figures 2–7. Separation of the tetrad

In text figure 3 the tetrad has split at each end and the splits are at right angles to each other. To the right the homologues are separated by the primary split of Wenrich. To the left is shown the secondary split, separating the fused daughters of homologues. If these splits be continued until they intercept each other, there will result a condition like text figure 4, in which one chromatid from each chromosome appears to cross over and unite with the other chromatid from the other chromosome. If we suppose the primary and the secondary splits to be widened so that the ends of the chromosomes are pushed apart until they all lie in the same plane, there will be formed a cross like text figure 5. The horizontal arms of the cross are homologues. The vertical arms are fused daughters of homologues. The spindle-fibers may be supposed to attach to the middles of the chromatids. If the chromosomes of text figure 5 separate in a horizontal direction the division will be reductional. If they separate vertically the division will be equational. Text figure 6 represents an equational division. Text figure 7 represents a simple crossing of two equationally divided chromosomes.

It now becomes evident that it is impossible to determine in Culex pipiens whether the first division is reductional or equational. In the first place the members of the pairs as they appear in late prophase of the first spermatocyte may represent homologues or they may represent fused daughters of homologues.

Figure 48 shows the three pairs at a somewhat later stage than figure 39. Where the members touch each other there may be a simple crossing like text figure 7, or a separation in different planes like text figure 4.

In figure 39 the ends of the larger pairs are united. It is impossible to tell whether the split which separates the middles is primary or secondary. In later stages appearances suggest very strongly alternations of splits at various points of the chromosomes. Where this occurs the chromatids may be said to be 'changing partners.' In figure 49, from another cell of the same cyst as figure 48, points *a*, *b*, and *c* are clearly simple crossings of the fibers; point *d* suggests very strongly a 'change of partners.'

Figure 50 is a later stage in which each of the three pairs shows a change of partners at one end. In pair *a* this has progressed well toward the middle. In this and the succeeding figures, pair *b* is the small pair. Figure 51 shows that the chromatids of pair *a* are changing partners at both ends, thus forming a ring with a cross at each side. The difference in length of the transverse arms of the cross is a matter of perspective. Figure 52 shows the three pairs, each with a change of partners at one end. In *a* the split of the ends is in the plane of the paper and the lower point of union is bent down so that the chromatids uniting there appear shorter on account of perspective.

In figure 53 each of the three pairs has formed a ring, with a change of partners at each end. This is a typical form of prophase tetrad. The tetrads have been displaced for clearness in drawing. The direction and extent of displacement is represented by the direction and the length of the arrows.

In figure 54 each of the three pairs has formed a cross by a change of partners comparable to text figures 5 and 6. This is also a typical form of prophase tetrad. Tetrads of either the ring or the cross variety may thus be formed by any one of the three pairs.

In figure 55 *a* and *b* are tetrad crosses, while *c* has the members still separated. In figure 56 *a* and *b* are rings, the latter, however, incomplete at the lower focus. Tetrad *c* is a cross seen from side view.

The change from late prophase to metaphase and anaphase is passed through by each tetrad independently of the others. In figure 57 tetrads *b* and *c* are in early anaphase while *a* is still in prophase. The prophase tetrads are thicker and less clean-cut than those in metaphase. This is probably due to relative degree of condensation.

In figure 58 *b* and *c* are in late metaphase, showing clearly their fibre attachments. Tetrad *a* is a thick, irregular prophase cross without apparent fiber attachments. In figure 59 the small tetrad *b* is still in prophase, but the outlines are more clean-cut, showing a certain degree of condensation. In figure 60 the outlines of the cross-shaped tetrad *c* are smooth and a fiber attaches to one of its arms. Tetrad *a* has separated irregularly so that a part of the upper member is still attached to the member going to the lower pole.

In figure 61 all three tetrads are in metaphase condition. The small one, *b*, is shown at two foci.

Figure 62 is a late metaphase, and figure 63 a similar stage from a slide which was much decolorized. The separate chromatids are here plainly seen. Tetrad *c* strongly suggests text figure 6.

Figure 64 shows an early anaphase in which the small tetrad *b* has divided before the others.

The spindle fiber, wherever it can be distinguished, is always attached near the center of the dyads, consistent with the condition in the spermatogonia.

3. Later anaphases and telophases. When the dyads have separated in anaphase and are approximately at the poles, they split longitudinally, forming three pairs of V-shaped monads at each pole. In a side view of the anaphase spindle the chromosomes are often difficult to separate and inconvenient to draw, due to overlapping.

In figure 65, an oblique view of an anaphase, the dyads have already split into monads. The two poles of a slightly later stage are shown from polar view in figure 66. Figure 67 shows an optical section of a similar stage cutting through each arm of the monad V's. Such appearances are very characteristic of this stage.

Figure 68 is a slightly later stage when the chromosomes have reached the poles. The sister monads are drawn closely together, especially in their middles. At *a* is shown the upper pole; *b* is an optical cross section of the spindle fibers; and *c* shows the lower pole. The chromosomes at *c* are becoming somewhat vesicular at their ends. The sister monads tend to unite at this stage, beginning at the middle and progressing toward the ends.

Figure 69 shows five optical sections along the polar axis of a first spermatocyte telophase. The sister monads separated in the anaphase seem to have reünited in the telophase. In figure 69 *a* is at a higher focus than *b*. The numbers about the circumferences of *a* and *b* mean that the chromosomes opposite those in *a* are continuous with those numbered correspondingly in *b*.

The middles of the chromosomes are drawn together to the centre of 69 *a*. The distal ends of each pair are united as shown in *b*. Thus each united pair makes a complete ring by union of its distal ends. One of these rings is seen in chromosome 2 of 69 *b*. The lower focus of the middle (upper focus shown in *a*) forms the right side and the upper focus of the united ends forms the left side of this ring as shown in the illustration. Figure 69 *c* is an optical cross section of the spindle fibers; 69 *d* is the upper focus of the lower nucleus; and 69 *e* its lower focus. The corresponding chromosomes are again shown by the numbers. This nucleus has been rotated so that its chromosomes are not at right angles to those of the upper nucleus. The diffusion process has progressed farther, so that the chromosomes are very thin. They meet at the center of *e*, corresponding to the condition seen at *a*. The more distal part of the chromosomes is at *d*, and the sister monads of chromosome 3–3^1 seem to be separated. The distal ends of each are united, however, as in the upper nucleus, so that in this case also each chromosome forms a complete ring.

A side view of this stage is shown in figure 70 and a little later stage in 71. In figure 72 the daughter nuclei have become more nearly round and a small granule is seen at the distal end of each. The chromosomes are still heavily stained at the proximal ends.

In succeeding stages, as shown in figures 73, 74, and 75, the chromosomes become very diffuse, but do not entirely disappear. The granule becomes more conspicuous and a second smaller granule appears near it. These granules undoubtedly correspond to the nucleoli of the spermatogonia and the first spermatocytes. They will be called the larger and the smaller nucleoli.

The spindle fibers of the first spermatocyte telophase are at first widely separated, (figs. 68, 69, and 70). They gradually draw nearer together and appear very black. A constriction may be seen at their middles so that they often look like a shock of wheat (fig. 74). At a later stage they disappear. Black bodies are seen at the time of the dissolution of the spindle fibers, as shown in figures 73, 74, and 75.

The telophase nuclei become the interkinesis nuclei of the second spermatocytes.

d. Second spermatocytes

1. Interkinesis, prophases, metaphases, anaphases, and telophases. The young nucleus of the second spermatocyte appears as shown in figure 76. The chromosomes are diffuse and are connected with the two very dense nucleoli, one of which is always larger than the other. The larger nucleolus becomes diffuse (fig. 77) and disappears (fig. 78). Meanwhile the chromosomes thicken (fig. 79), and the smaller nucleolus, still densely staining, appears attached to the end of a diffuse chromatic thread. The small nucleolus then divides (fig. 80), as the chromosomes become very thick.

The separate pairs have not been clearly distinguished at this stage, but it seems probable that the small nucleolus is descended from the pair of terminal granules of spermatogonia and first spermatocytes. Evidence for this is shown in figure 79, where the small pair of chromosomes is separate from the other two at the top of the figure. The small nucleolus is therefore associated with the larger chromosomes.

As the chromosomes become condensed, the small nucleolus can no longer be distinguished and the three pairs appear as in figure 81. The small pair in this and the succeeding figures is lettered *b*. One member of pair *a* in figure 81 is foreshortened as a result of twisting. In succeeding stages the middles of the members of each pair touch and the ends radiate somewhat at right angles (figs. 82, 83, 84, and 85). Thus three crosses are formed which tend to occupy a peripheral position in the nucleus. The monads do not cross each other, but merely touch, so that the opposite arms of the cross are parts of different monads.

Figures 83, 84, and 85 are from picro-formol-acetic fixation and, as a result, the chromosomes are somewhat distorted. Figure 83 shows a trace of the nucleolus between the larger pairs. It is surprising that this should persist so late in this cell as it usually disappears before this time. The cross-shaped dyads next group themselves into a metaphase, the ends of the monads point toward the poles to which they are to go, and the middles still touch at the equator of the spindle (fig. 86). The fibers attach, as in previous stages, to the middles of the chromosomes.

As the chromosomes pull apart in anaphase (fig. 87), the ends tend to reverse their direction and to point toward the equator of the spindle. A later anaphase is shown in figure 88.

In the telophase (fig. 89) the chromosomes become diffuse, especially at their distal ends. In figure 89 the spindle fibers have been pushed out of place and the nuclei are at right angles to each other. At *a* the nucleus is shown in side view. At *b* is an optical transverse section. The chromosomes are plainly visible, but I was unable to count them on account of the small size of the nuclei. The telophase nuclei of the second spermatocytes become the nuclei of the spermatids.

e. Spermatids

The spermatid nuclei rapidly increase in size and the two nucleoli of unequal size again appear, with the diffuse chromosomes radiating from them (fig. 90). As the spermatid is changing into a spermatozoon, the chromosomes become more and more tenuous and the nucleoli become diffuse (figs. 91 and 92). One nucleolus can be seen after the other has disappeared (fig. 93). Figures 91, 92, and 93 are from a slide more darkly stained than figure 90. In figures 94 and 95 the staining is light and thus the spermatids do not show their chromosomes. The spermatid in figure 95 has begun to lengthen and is larger than that in 90. I judge it to be at a stage between figures 90 and 91.

f. 'Pathological' cells

Among the second spermatocytes and the spermatids cells are frequently seen which are evidently first spermatocytes which have failed to divide while the tetrads have divided. The division of the tetrad into its four monads is sometimes normal as in figure 94, but more frequently abnormal. In figure 95, *a* and *b* have each divided into four monads. Tetrad *c* has formed a cross, the longer arms of which have broken off, and one of these has divided,—*c'*. In other cases the division is so irregular that it is impossible to determine the origin of the different elements. This karyokinesis without cytokinesis occurs very frequently in some individuals, not at all in others. When the division of the tetrads is normal, we thus have a cell with twelve chromosomes.

A type of cell, rarely found by me, corresponds to the pycnotic type of degeneration described by Lomen ('14). The nuclear matter seems very much condensed and the cytoplasmic area is very clear. These cells occur in various places in the testes of a few individuals and usually near the walls. Figure 96 is a drawing of two of these.

Another type of degeneration mentioned by Lomen ('14) and observed in my material is much more frequent than the pycnotic. In these cells the nuclei become very large and take a blue-black stain with iron-haematoxylin. They seem to absorb all the cytoplasm and to swell up to many times the size of the original cells. Very dark masses may be seen in these nuclei. Cells of this sort occur among the spermatogonia and they retain their form without disintegration down among the spermatids. They are more frequent in some individuals and do not occur at all in others. Figure 97 shows such a cell accompanied by a first spermatocyte nucleus lying alongside it and at a lower focus. The difference in the size and the staining-reaction of the two is very striking. The first spermatocyte shows its nucleolus containing four darkly stained areas, as is usual.

B. OVARIAN MITOSES

The ovogonial chromosomes appear in all essentials like the spermatogonial. Likewise the mitoses of the follicular cells of the ovary are not essentially different. Late prophases show three pairs, (figs. 98 and 99) one pair, *b*, smaller than the other two, *a* and *c*. One of the larger pairs, *c*, frequently shows two granules at one end. These may be comparable to the terminal granules of spermatogenesis or they may be sex elements. They are somewhat larger than the terminal granules usually are, but the latter vary so much, both in size and in staining reaction, that I have been unable thus far to reach any decision regarding their character.

In one ovary two cells in late prophase were found which had the chromosomes split longitudinally. The small pair from one cell with each member split is shown in figure 100. The three pairs from the other cell are given in figure 101. This condition seems to indicate the double nature of each of these chromosomes at this stage, although the separation of the elements is probably the result of technique.

Figures 102 and 103 show metaphases in stage A. In the latter cell were counted as many as eight diffuse bodies, resembling the larger nucleolus, but distributed about in the cytoplasm near the chromosomes. In figure 104 the chromosomes are beginning to assume the parallel arrangement of stage B shown in polar view by figure 105. An equatorial view of the same stage along the parallel axis is shown in figure 106.

Figure 107 shows an early, and figure 108 a later anaphase, from two sections. The separate elements are not distinguishable at pole *a* of figure 108, but pole *b* shows six V-shaped chromosomes lying parallel to each other. Figure 109 is an anaphase with the members of the pair to the right fused. The chromosomes are approximately parallel, showing that they have been pulled out from the parallel arrangement of the metaphase plate. In figure 110 the chromosomes are crowded at pole *a*, separate and parallel at pole *b*. Figures 107, 108, 109, and 110 are from picro-formol-acetic fixation, which accounts for the fusion and the contraction of the chromosomes. Figure 111 shows an oblique view of an anaphase from two sections. An optical section is drawn at pole *a*, while the six chromosomes are shown entire at pole *b*.

Figures 98 to 106 are ovogonial; figures 107 and 111 are follicular; while the nature of the tissue from which figures 108, 109, and 110 were drawn is doubtful.

C. MITOSES OF THE THORACIC HYPODERMIS

The developing limb buds and wing buds of the thoracic hypodermis of the larvae show numerous mitotic figures. Three pairs of chromosomes appear in late prophase (figs. 112 and 113), one pair, *b*, smaller than the other two, *a* and *c*. Metaphases stage A, (figs. 114 and 115) and stage B (figs. 116 and 117) are similar to the corresponding stages of spermatogonial and ovarian mitoses. Figure 118 is a side view of a chromosome splitting in early anaphase and figure 119 shows the six chromosomes lying parallel to each other as they go towards the poles. The chromosomes in figure 119 are V-shaped but only one arm of each V can be shown. The relatively greater tendency of the chromosomes to appear fused in the drawings from hypodermal cells is due to the fact that fixation is interfered with by the chitinous part of the integument. This will be discussed more fully under Technique. More detailed description of the somatic complexes will appear in papers by Mr. Hance and Miss Holt.

D. SUMMARY OF OBSERVATIONS

The chromosomes of Culex pipiens are six in number in all diploid mitoses. At all stages the homologues tend to be approximated in three pairs. One pair is smaller than the other two.

As the chromosomes of diploid cells are passing from prophase to anaphase, they occupy a flat metaphase plate. Two successive stages, A and B, are distinguished in the metaphase. In stage A the chromosomes are bent rods, swollen at the ends and constricted in the middle. The middles tend to be nearer than the ends to the centre of the plate.

In stage B the chromosomes tend to be of more uniform diameter. The pairs assume an arrangement parallel to each other, which is continued into the anaphase. Division of each chromosome begins at the middle and the daughters pass to the poles as V's.

In the late prophase of the first spermatocyte the approximated pairs become crosses or rings. The chromatids of each member of each pair separate at one or both ends and each unites with a chromatid from the other member. When this occurs at one end a cross is formed; when at both ends, a ring. A process comparable to this occurs at earlier stages in which the chromatids 'change partners' in part or all of their length. It is thus impossible to say whether the first spermatocyte division is reductional or equational.

In the second spermatocyte prophase the chromosomes appear as three pairs of rods, the members of which separate in anaphase. Three chromatids go to each spermatid.

The spindle fiber attachment is approximately at the middle of all chromosomes. In the tetrads it is attached to the middle of each chromatid.

A distinct nucleolus always appears between successive diploid mitoses and in the growth stages of the first spermatocyte. It often shows four darkly stained regions. These nucleolar elements are separated at certain stages. Two of them appear to form terminal granules on one of the larger chromosome pairs, while the other two are connected with the middle of the same pair.

In the second spermatocyte interkinesis and in the spermatids, two nucleoli of constantly unequal size appear. In the second spermatocyte the smaller nucleolus becomes the terminal granules of one of the larger pairs, while the larger nucleolus becomes diffuse and disappears.

III. DISCUSSION

A. INTRODUCTORY

It will now be of interest to consider the causes for the wide difference of opinion prevailing among the investigators of Culex pipiens. This diversity is not merely a matter of interpretation; it concerns facts. The number of chromosomes, the normal occurrence of synizesis, the nature of synapsis, as well as the much discussed matter of chromosome individuality are all questions in regard to which the observations seem to differ.

B. TECHNIQUE

a. Experiments and observations

Among all of the causes of difference of observation and interpretation in cytology, the matter of technique is of prime importance.

The class in cytology under Dr. McClung undertook during the winter of 1914–15 to investigate Culex pipiens. The abdomens of larvae and pupae were cut off near the thorax and the tips of the abdomens also removed for better penetration. In some cases parts of the thoraces of larvae were also preserved. Division figures were found, not only in the gonads, but scattered through various parts of the somatic tissue as well. They were especially abundant in the developing leg and wing buds of the thorax, and were also found in the nervous system. In certain of the pupae the degenerating larval intestines also showed division figures, mostly multiple complexes.

The best fixation seemed to be given by a formula of Dr. Ezra Allen, consisting of saturated aqueous picric acid, 75 per cent; formol, 15 per cent; glacial acetic acid, 10 per cent. The results, however, were not very satisfactory, which seemed to be due in part to slow penetration, in part to the distorting action of the fixative. Flemming's fluid has been employed by Metz in his investigations on the Diptera. It does not give good results, however, unless the tissue to be fixed is dissected out in minute pieces and immediately placed in the fixative. An attempt to fix in Flemming without dissecting out the tissue resulted in shrunken and blackened sections, clearly the result of poor penetration and delayed fixation.

At the suggestion of Dr. Metz and Dr. Wenrich, therefore, in the fall of 1915, I dissected out the gonads of larvae and pupae in tap water and transferred immediately to strong Flemming. The gonads were drawn up in a capillary pipette and transferred to the fixative. After some experimenting it was found that the dissection may be done with greatest facility by cutting off the tip of the abdomen just posterior to the gonads and then pressing out the latter.

The gonads were fixed from two to twenty-four hours in small vials. The fluid was then replaced by water in which the tissue was left over-night. The water was then gradually replaced with alcohol until the percentage reached 95, in which the tissue was stained with eosin for the purpose of orientation in the paraffin. Clearing was done in aniline oil followed by bergamot or xylol and when all the water and alcohol were removed the gonads were transferred from the vials to melted paraffin. About one hour sufficed for thorough infiltration. Sections were cut 6μ in thickness and Heidenhain's iron-haematoxylin was used with no counterstain. A few testes were dissected out and fixed in Allen's picro-formol-acetic. This gave fair fixation but the chromosomes appeared much shorter and thicker and had a greater tendency to fuse. A few abdomens of adult females were torn to pieces in Flemming's fluid and good fixation of dividing figures was obtained.

It was found to be difficult to obtain good penetration of thoracic hypodermis. In the larvae this tissue is full of division figures, but the chitinous epidermis, binding so closely on the outside, and the adipose tissue, adhering on the inside, seem to prevent the proper penetration. Probably the fat takes up the osmic acid, as it appears much blackened. The attempt was made to remove the epidermis, and although the outer layer was removed, the inner layer still interfered considerably with penetration. By considerable searching, however, it was possible to obtain a few good figures at the edge of the tissue. Mitoses nearer the center were usually much distorted, and the chromosomes lying near each other were fused.

All degrees of fusion were observed in the chromosomes, corresponding to the distance of the cells from the cut edge of the tissue. This fusion is very striking in picro-formol-acetic. Often each pair appears like one chromosome, so that there will be only three chromosomes instead of six. In tissue put up in picro-formol-acetic without dissecting, spermatogonial premetaphases often appear so distorted that they fuse into a triangle, each corner of which corresponds to the approximated ends of two pairs. The chromatin may then become aggregated at the corners so that three masses are formed, each connected to each of the others by two strands, corresponding apparently to the middles of the original chromosomes. These strands appear at times to have been broken and the three masses to have rounded off into three smooth round 'chromosomes.' All sorts of irregularities of the process, as would be expected, occur. The prophase chromosomes assume all sorts of irregular shapes and the entire contents of the nucleus may be shrunken into a 'synizetic' mass.

In gonads dissected out and fixed in Flemming the fixation is not by any means at all times perfect. This may be judged in general by the appearance of the tissue as a whole, whether shrunken or not. In some cases, however, the chromosomes appear well fixed, while the tissue may exhibit some degree of shrinkage. Figures 17, 18, and 19 are from one slide in which the general fixation was not good. The homologues are more or less shrunken and appear to touch each other. Figures 16, 20, and 21 exhibit the same stage with good fixation. The chromosomes are well separated.

Under normal conditions it is questionable whether the chromosomes ever touch each other during mitosis. The parallel union of the members of pairs in first spermatocyte telophase may be an artifact (figs. 68 and 69). In the early telophase of the spermatogonia (fig. 28), the homologues remain distinct.

The chromatin of the mosquito seems at times to have a tendency to flow together. In the cross-shaped tetrads the strands connecting the arms about the opening at the centre would be expected to be of approximately equal thickness—the width of a chromatid. Occasionally one or more of these strands may be reduced to a mere shred, even in prophase, before we would expect any tension upon it. Such a condition is shown by tetrad *b* of figure 54.

The contrast between the sharp outlines of the Flemming material (fig. 82) and the picro-formol-acetic fixation of the same stage (figs. 83, 84, and 85) is obvious. In this case the gonads fixed in the latter were dissected out before fixation. The conditions are therefore much better than in those not dissected, but 'synizesis,' nevertheless, occurred. The fusion of the chromosomes in the picro-formol-acetic material is seen in figures 107, 108, 109, and 110.

b. Review and criticism of literature

It will now be of interest to consider the technique employed by the different investigators in the study of the chromosomes of Diptera.

Stevens ('09) "removed the anterior segments and secured good fixation in Flemming's fluid and fairly good in Gilson's mercuro-nitric." Most of her work was done, however, with aceto-carmine which tends to swell the tissue. The gonads were dissected out for the aceto-carmine method. The errors in Stevens' work are few and her results more consistent than either Taylor's or Lomen's.

Lomen ('14) simply cut the larvae, pupae, and adults in the middle and fixed in from 8 to 9 parts corrosive sublimate, 3 to 4 parts absolute alcohol. To 96 parts of this were added 4 parts of glacial acetic acid. This was called S-A-E. Zenker's potassium-bichromate, sublmate, acetic, K-B-S-E, was also used and was considered better for dividing figures. Lomen's figures are all very diagrammatic.

Taylor ('14) tried aceto-carmine for which she dissected out the gonads. She states, however, that it is not permanent, not good for finer details, nor for somatic mitoses. For material to be sectioned she used as fixatives, Benda's fluid, acetic-bichromate, Gilson's mercuro-nitric, Flemming, and Gilson-Petrunkewitch. She does not state whether she dissected her material to be sectioned, but since she laid such stress on somatic mitoses, it is probable that she kept her tissue largely intact. Her figures are consistent with each other neither in number of chromosomes nor in their form. They suggest very careful drawings of poorly fixed material.

Metz ('14) has made use of aceto-carmine preparations and Flemming material. In all cases the gonads were dissected out before fixation. The chromosomes stand out clearly and are separate from each other except in figures 9, 15, and 16 of 'metaphases' in which the homologues are in contact. This 'synapsis' as Metz calls it, I believe to be an artifact.

C. SYNIZESIS

The word synizesis was coined by McClung ('05) to denote a "condition of the nucleus in which the chromatin is found massed at one side of the vesicle, without regard to whether it is a normal phenomenon or not." Synizesis is supposed usually to take place at the beginning of the growth period. The word synapsis has been used by many writers to denote this condition, but it is clear that Moore ('95), who coined the latter term, intended it to be used for the pairing of the chromosomes before reduction. This will be treated more fully under Synapsis.

The existence of synizesis, as a normal phenomenon, is questioned by many. In the case of the eggs of Paludina, however, Popoff ('08) observed it, and concluded, as a result of his measurements, that it is normal. There is no contraction of the chromatin, but rather a rapid increase of the nucleus in size, due to osmosis. This is not immediately compensated by expansion of the chromatin, and thus an appearance of synizesis is produced. Much the same phenomenon was observed by Lawson ('10) in plants and demonstrated by measurements.

Stevens observed synizesis in Culex ('09) and states that "the granular and beaded chromatin threads are wound about a large nucleolus." She considers that it bears no relation to synapsis but that it is a period of reconstruction. In Musca domestica ('08), however, she states that it does not occur.

Lomen ('14) is very skeptical of its being a normal phenomenon since he obtained it with certain fixatives but not at all with others. K-B-S-E, he states, gave it, but not S-A-E.

Taylor ('14) seems to contradict herself in her statements about synizesis. She considers that it marks off the spermatogonia from the first spermatocytes. When she finds it in very young larvae she supposes that they are precocious in maturation, but when she finds it in somatic cells (p. 391) she concludes that it is not significant in Culex as it is in other creatures. Finally, (p. 394), it is concluded that synizesis represents an inactive phase of the nucleus. This last statement is probably true in a different sense from what Taylor intended.

In the Hemiptera Wilson ('12) observed a distinct synizesis occurring at the time of synapsis and is inclined to consider it a normal phenomenon. In speaking of "the polarized amphitene, or 'bouquet-stage,' that is characteristic of Tomopteris, Batracoseps and other forms," Wilson states that it is "entirely wanting in these insects, where, in its place, we find the closely convoluted and apparently non-polarized synaptic knot, or synizesis. The controversy as to whether the latter is an artifact, due to the coagulating effect of the reagents seems to be terminated by the fact . . . . that the synizesis may be seen in the fresh (living?) material immediately after gentle teasing apart of the cells in a normal fluid (Ringer's solution) in which the spermatozoa continue actively to swim." Wilson seems to question just how much alive "the fresh (living?) material" may be, and might also have questioned the perfect normality of Ringer's solution. It would seem that during the synaptic phase the chromatic elements are in a very unstable condition, and that any shock is likely to cause them to clump together.

Whatever may be the normal condition in other forms, I am convinced from observations with different methods of technique, that in Culex pipiens synizesis is an artifact.

D. CONTINUITY OF THE CHROMOSOMES

The matter of chromosome continuity has been ardently assailed by some investigators and as ardently defended by others.

It is tacitly held for Culex pipiens by Stevens and Taylor. Lomen ('14) considers the matter at length and concludes that all appearances are against it. He has, in first spermatocyte growth stage, a 'Brockenstadium' in which the chromosomes are completely broken up. The chromatin appears as fine granules which collect into threads at a comparatively late stage. A comparable condition occurs between successive diploid mitoses.

I have not been able to count the number of chromosomes in all stages between telophases and early prophases, but I have nothing at all suggesting such a 'Brockenstadium' as Lomen figures. Fine chromatic threads are visible in all 'resting' stages in my material. They occur in the earliest first spermatocyte nuclei (figs. 29, 30, and 31), as well as in all stages from first spermatocyte telophase to second spermatocyte prophase (figs. 69 to 80). I therefore conclude that the chromosomes are continuous between mitoses and that Lomen's 'Brockenstadium' and 'Verstäubungsprozess' are artifacts.

E. CHROMOSOME NUMBER

Stevens ('09) concludes that, in diploid mitoses of Culex pipiens, each of the six chromosomes divides longitudinally, and six go to each pole. Three is the reduced number appearing in both maturation division.

Taylor ('14) considers three as the somatic number. She suggests that there may be but one maturation division, which is equational. This, she recognizes, would involve the non-participation of one of the gametic nuclei in fertilization. She suggests, as an alternative, that the homologues have fused in her material, thus reducing the number. She accounts for her disagreement with Stevens by supposing that the latter worked on Culex pungens. Stevens has used the names pungens ('09) and pipiens ('11) interchangeably. They appear to be the same, both taxonomically and cytologically. Taylor considers that one member of each pair appearing in diploid metaphase goes to each pole. The homologues are thus interpreted by her as daughter chromosomes.

Lomen ('14) has three chromosomes going to each pole in all anaphases, and three chromosomes appearing in prophase. But his spermatogonial anaphase shown in his figure 10 could as well be interpreted as having six. The prophase chromosomes each split transversely to form the six metaphase chromosomes.

But the only evidence that Lomen has for the cross-splitting is a slight constriction and bending of the prophase chromosomes in the middle. He states that Dr. W. Stempell, his professor, is inclined to consider six as the normal number, and concludes that therefore reduction must take place in the growth period. This, however, is inconsistent with his account of spermatogonial anaphases which, he states, show only three, and therefore reduction would be unnecessary.

From what I have said in regard to the importance of employing the best technique, it will be evident, I think, that the inconsistencies of the results of Taylor and Lomen are largely due to imperfect technique.

In a brief paper on Corethra plumicornis, Dehorne ('14) concludes that three is the diploid as well as the haploid number of chromosomes in that species. Only one figure is shown, very diagrammatic, of a somatic prophase. Dehorne speculates about the possibility of parthenogenesis as an explanation of the reduced number. It seems probable to me that here, also, the close approximation of homologues is the true explanation of the condition, and that a refinement of technique would reveal this.

F. NUCLEOLI, HETEROCHROMOSOMES, AND CYTOPLASMIC BODIES

a. Introductory

The occurrence of condensed bodies in resting nuclei and of darkly stained bodies in the cytoplasm has been the cause of much confusion and speculation in the history of cytology. It must be confessed that but little progress has been made in regard to our knowledge of the cytoplasmic bodies. The nucleoli, however, are somewhat better understood. In general they bear a staining reaction reverse to the chromosomes. When the chromosomes stain darkly the nucleoli are very diffuse or absent (?). When the nucleoli come into prominence, the chromosomes become diffuse or seem to disappear. For this reason it has often been assumed that chromatic matter passed from chromosomes to the nucleoli and the reverse. But there is no more reason to suppose that this takes place than that certain changes are going on in each that make them darkly-staining at one time, not at another. That the latter theory is preferable would seem to be the case in the chromosome-nucleoli. In many forms the unpaired or accessory chromosome appears condensed in the resting nucleus, but becomes diffuse in metaphase or anaphase. Other odd chromosomes or parts of chromosomes condense when the others are diffuse.

b. Heterochromosomes

Stevens ('11) found in Anopheles an unequal pair of chromosomes attached to the smaller pair of euchromosomes in spermatogonial metaphases and in first spermatocytes. In ovogonia the corresponding elements are each equal in size to the larger member of the unequal pair. In resting spermatogonia the condensed pair is associated with a plasmosome and in growth stages the two members can often be distinguished. They are condensed and attached to the still diffuse smaller pair of euchromosomes. The first maturation division separates the members. The interkinesis condition was not made out, but the dimorphism of the spermatids was declared evident, each having a single nucleolus, which might be either large or small.

Unequal *xy* pairs were found by Stevens ('08) in nine Myodaria, unassociated with the euchromosomes. The larger member of each pair was shown to be the sex chromosome by comparison with the female in Musca, Calliphora, Sarcophaga, Scatophaga, Tetanocera, and Eristalis, but not in Lucilia and Phorbia. It is probable that the same condition obtains in the latter two genera as well; but in Drosophila the reverse seems to be the case according to Bridges ('16). Stevens supposed that in Drosophila the larger bent member was the *x*-element.

Stevens ('09) has made considerable search for heterochromosomes in Culex. Especial attention was given to the smaller pair, as it seemed to condense at some times earlier than at others. I have found no evidence for this in my material. No inequality could be found by Stevens.

My own evidence in regard to heterochromosomes in Culex is not at all conclusive and applies to only a few of the spermatogonial metaphases and one prophase. By reason of the twisting and the irregularity of the chromosomes at this stage and because of the variable number of diffuse bodies of similar appearance it has thus far been impossible to gain conclusive evidence. If, however, as figures 12 and 14 seem to indicate, we have a diffuse body attaching to one member only of one of the large pairs, we have here a possible sex element and a condition comparable with that of Ascaris as shown by Herla ('95) and Frolowa ('13).

c. Nucleoli

The plasmosome of Culex was noticed by Stevens ('09) and was thought to absorb chromatin extruded by the spireme.

Lomen ('14) noticed a clear space about the nucleolus and concluded, therefore, that the spireme is not at all connected with the latter. He considers that the variations in form of the nucleolus are degenerative. He finds only one in second spermatocytes, but occasionally two in the spermatids.

I have nothing at all to offer as an explanation of the nucleolar structures in Culex, but I believe that they all arise from a single pair of chromosomes. In spermatogonia and first spermatocytes they never show more than four elements, two of which, the terminal granules, are attached to the ends, and the other two, the 'plasmatic' elements, are connected with the middles of each member of one of the large pairs. Whether this is the same pair that is associated with the 'sex-element,' I am unable to say. The greater size of the plasmatic elements in relation to the terminal granules, and the more precocious tendency of the former to become diffuse, lead me to identify them with the larger nucleolus of the interkinesis nucleus and of the spermatids, while the terminal granules evidently form the smaller nucleolus as shown in the figures.

G. METAPHASE—STAGES A AND B

The peculiar parallel arrangement of the pairs of chromosomes, as in stage B of the metaphase of Culex, has not been noticed, so far as I am aware, in any other form. There are suggestions of it in some of the figures in other works upon Diptera, but it is nowhere else shown that the chromosomes begin their division only after assuming this arrangement. In diploid complexes of Drosophila ampelophila, Bridges ('16) has shown a splitting at the ends of the chromosomes. The spindle fiber is median, as in Culex, but the spermatogonial and ovogonial divisions begin by a split at the ends of the chromosomes and "the separation at the point of attachment does not take place until the elongation of the cell." If we may judge from the few figures given, the chromosomes which are splitting appear to take on a parallel arrangement. In spermatogonial divisions of Culex this split at the ends does not occur, but it does occur occasionally in the somatic cells. The division of the somatic chromosomes, however, seems to be from the middles, comparable with the divisions of spermatogonia and ovogonia. These matters are to be discussed more extensively in a paper by Mr. Hance.

It has been noticed that, in well fixed material, the condensed chromosomes act as if they repelled each other. In second spermatocyte late prophases (figs. 82, 83, 84, and 85,) and metaphases (fig. 86) the ends of the chromosomes are far apart, while their middles appear as if tied together by the spindle fibers. This condition may also be seen in the tendency of the outer members of the pairs in diploid metaphases to bend away from the inner members at their ends while their middles are more approximated, as if pulled together or toward the center of the metaphase plate by the spindle fibers. In spermatogonia (figs. 11, 12, 13 and 14), this is striking, and is even more so in the actual preparations, in which differences of vertical focus are evident.

As the chromosomes condense and stiffen, the tendency is for the pairs to assume a parallel arrangement apparently under the influence from the tension of the spindle fibers. Figure 14 looks very much as if pair *a* would be pulled about beneath *b* and *c* and come up between the two and parallel to them. This appears to be taking place in figure 15, in which I have lettered the pairs in a comparable manner. The result would be a parallel arrangement as shown in the succeeding figures. In figure 16 this process is not completed, and the chromosomes are more irregularly bent. Figure 20 shows a typical case in which the rods are comparatively straight. The ends of pairs *b* and *c* are somewhat bent outward as if they were repelled from the other pair. There seems to be considerable tension on the middles of the chromosomes which are beginning to divide.

Whether this be the explanation of the assumption of the parallel arrangement of the chromosomes or not, it nevertheless remains true that this position assures the middles getting as near the center of the plate as possible while the chromosomes are stiff and lie in one plane.

H. PSEUDOSYNAPSIS, SYNAPSIS, AND HEREDITY

The term 'Synaptic Phase' was first used by Moore ('95) who speaks (p. 284) of "the Rest of Transformation (Synaptic Phase) between the First and Second Spermatogenetic Periods." He states (p. 276), "the cellular generations of spermato- or ovo-genesis before and after the numerical reduction of the chromosomes will be distinguished as those of the first and second spermato- or ovo-genetic series." Under the heading "the Rest of Transformation" (p. 284) Moore considers the entire period from the last spermatogonial division to the formation of the tetrads. "The nuclear threadwork again grows coarser and thicker, displaying at the same time a peculiar tendency to contract to one side of the nucleus, leaving a great clear space across which stretch numerous linin filaments. The contraction is not so marked when the cells have been preserved with osmic acid, nor on the outside of sections which have been preserved with Flemming's fluid, where the osmium has acted directly upon the cells." "Whether it exists in nature or not, the cells display at this period, and at no other, a remarkable tendency to have their chromatin contracted in consequence of some internal change which renders these nuclear figures diagnostic of the particular period in question."

It is obvious then that Moore questions the normality of the contraction, nor does he consider the contraction the essential or important phenomenon of this period. It is merely 'diagnostic.' That the numerical reduction of the chromosomes is the essential feature appears from page 287. "There are thus, after the rest of transformation, only half as many chromosomes, i.e., separate chromatic masses, as there were before, and the halving of their number, being brought about while the nuclei are still at rest, is to that extent comparable to what is now known to go forward during the maturation of the reproductive elements of plants. I therefore propose the term Synaptic phase to denote the period at which this most important change appears in the morphological character of reproductive cells."

Again, in 1905, Farmer and Moore, consider the question of reduction. The term synapsis is derived from the Greek *συνᾱπτειν*, to fuse together, and is used to refer to the union of the homologous chromosomes rather than to the contraction of the entire chromatin content. "The synapsis represents that series of events which is concerned in causing the temporary union in pairs of pre-maiotic chromosomes, previously to their transverse separation and distribution, in their entirety, between two daughter nuclei." Synapsis takes place then in the first gametocyte nucleus, in the rest of transformation.

The terms 'maiosis' or 'maiotic phase,' from the Greek *μείωσις*, reduction, are used "to cover the whole series of nuclear changes included in the two divisions that were designated as Heterotype and Homotype by Flemming."

Farmer and Moore subscribe to telosynapsis and prereduction. In discussing the spermatogenesis of an Acridian, Wenrich ('16) demonstrates parasynapsis and reviews the work of other investigators upon other forms. In the Acridian the chromosomes enter the synaptic period as single leptotene threads. The homologues conjugate parasynaptically during the zygotene stage, and at the end of the growth period a secondary split at right angles to the original plane of separation becomes evident. Four chromatids are thus demonstrated which are the four elements of the tetrad.

It would seem in general, from the more recent work, that parasynapsis is at least the more usual mode of pseudoreduction and that either maturation division may be reductional, depending upon the species or upon the particular chromosome. Wenrich has shown that a single chromosome may divide reductionally either in the first or in the second maturation division and that this is governed by chance. The possibility of determining this was due to a morphological dissimilarity of the homologues. He concludes, in general, however, (p. 105), "As to which of the two maturation divisions is equational and which is reductional, no absolute rule can be laid down. The evidence, however, points to the probability that generally chromosomes with terminal spindle fiber attachment are not separated from each other until the second division, while those that have a non-terminal attachment are separated in the first, and that consequently in the former the reduction occurs at the second division, in the latter at the first division."

In the work on Diptera the term synapsis is used with such a variety of meanings that the whole matter is quite bewildering.

Lomen ('14) uses the term in what he considers to be Moore's sense to denote a crowding together of the chromatin at the beginning of the growth period.

Taylor ('14) avoids all trouble by using Haecker's ('11) "word 'syndesis' for the conjugation of the chromosomes, and 'synizesis' for their clumping together."

Stevens ('09) considers that "in Culex it is quite certain that parasynapsis occurs in each cell generation of the germ cells in the telophase." She uses the term synapsis to apply to the first spermatocyte metaphase and states that there is here a prolonged parasynapsis ending with a telosynapsis before the chromosomes separate. This is clearly a misuse of the term.

Metz ('14) uses the term synapsis to denote the association of homologues in metaphase and anaphase of diploid mitoses. In a short review ('16) he states that he has found this pairing of homologous chromosomes in all diploid cells of seventy-five species of Diptera, from the lowest to the highest of the order, and beginning in the egg before cleavage is completed.

Overton ('09) has shown this approximation of homologues in somatic cells of plants and reviews the literature dealing with this matter.

This approximation of homologues in diploid cells is evidently something very different from synapsis in the true sense, which is restricted to the first gametocyte. In the former case there is no reduction in number of the chromosomes which appear for the next mitosis. In the latter case we have pseudoreduction which persists until they are separated either by the first or by the second maturation division. This was expressed by Stevens ('08 p. 373) who, however, uses the term synapsis for pairing in diploid cells. "In the spermatocyte we get complete synapsis and reduction; in the spermatogonium, only a foreshadowing of reduction, and abundant proof that synapsis is here a side-to-side pairing of homologous chromosomes."

I would suggest therefore that the term synapsis be retained for the uniting of the homologues in the first gametocyte, and that approximations or brief temporary unions in diploid cells be called 'pseudosynapsis.' The fundamental difference between pseudosynapsis and synapsis is that the former results in no permanent reduction, while the latter results in not merely a pseudoreduction, a reduction in number, but ultimately in a qualitative reduction, a separation of parental elements, which may not take place until the second maturation division.

The term 'synaptic phase' was employed by Moore to denote the rest of transformation. The conditions have now been more closely analysed (Wenrich, '16) and it is shown that the uniting of the chromosomes, synapsis, takes place at the zygotene stage and that the homologues are not permanently separated in some cases until the second maturation division. I therefore use the term 'synaptic condition' for the paired condition of the homologues, which, in most forms, extends from the time of synapsis in the zygotene stage to the final separation of the homologues in the anaphase of the first or the second maturation division. We have seen that in the Diptera a synaptic condition occurs in diploid cells also, but that this is not brought about by a true synapsis, since it results in no permanent numerical or qualitative reduction.

It is while the homologues are in the synaptic condition that the closest union of parental elements takes place and this seems to be of considerable importance in heredity. In hybrids the development is often normal up to this time, apparently because the chromosomes from the different species remain distinct. But when the chromosomes try to unite in synapsis they are unable to do so in a normal manner and greater or less sterility results. This has been shown by Guyer ('00) in hybrid pigeons, by Federly ('13) in moths of the genus Pygaera, and by Wodsedalek ('16) in the mule.

As has been pointed out by Metz ('16) there is a union of the homologues in Diptera before cleavage is completed. This may account for the sharp delimitation of species and the non-occurrence of hybrids in this group, for, if matings of different incipient species took place, it is possible that the early cleavage cells resulting might become pathological through failure of the chromosomes of different origin to unite properly in pseudosynapsis. Thus the species would be separated in their incipiency and tend to diverge under the influence of this 'physiological isolation.' In the Lepidoptera pseudosynapsis has never been observed, and hybrids and species-intergrades are common, as appears from taxonomic work and especially from the work of Harrison and Doncaster ('14).

It is probably during the synaptic condition in the first gametocyte that factors brought in by homologous chromosomes have an opportunity to 'cross-over.' The synaptic conditions, in forms in which partial coupling occurs, have never been investigated. In plants a number of cases of partial coupling occurs and it appears that this is partial in the formation of both male and female gametes. Bridges ('14) has summarized some of this data from Bateson and from Gregory and shows that the linked factors of the sweet pea and of Primula may bear a linear relationship to each other. Castle and Wright ('15) also have shown that in the rat linkage is partial in both sexes. Sturtevant ('15) has pointed out that in the silk-worm moth, the experiments of Tanaka show that linkage is complete in the female, but partial in the male. The extensive work on Drosophila by Morgan and his students has shown that linkage is absolute in the male but partial in the female, and that the coupled factors bear a linear relation to each other.

Both genetic and cytological studies show that in the Lepidoptera the female is digametic, the male monogametic, while the reverse condition obtains in the Diptera. This, however, seems to have nothing to do with partial coupling which occurs in both sexes of the rat.

Thus far results from Primula and sweet peas (Bridges '14) and extensive evidence from Drosophila show that where partial coupling of three pairs of factors occurs, the factors stand in linear relationship to each other and that they are scattered, as if by chance, along a line, not collected together into groups. Moreover the factors which are very closely coupled are still in linear relationship, so that it would seem necessary, as Morgan and his students have done, to suppose that there is a transverse break in a pair of chromosomes and a re-fusion in such a manner that the broken end of each of the original members is united to the broken end of the other.

This theory, known as the chiasmatype theory, was first advanced by Janssens ('09) who suggested that it might explain genetic results. He supposed that the early tetrads twisted spirally and that homologous chromatids touched each other, broke at the point of junction, which was called the chiasma, and fused at this point with each other. Thus in text figure 4, B and C would undergo a break and re-fusion, while A and D would remain intact.

Janssen's investigations were done upon the amphibian Batrachoseps. As Wilson ('12, p. 442) says, his theory "gives the only simple mechanical explanation thus far offered as to how such an orderly exchange of materials may be effected." He is inclined to give credence to Janssen's results; but some doubt about the matter has arisen through more recent work. It is most important to investigate the conditions in some form in which the mechanism of heredity in regard to partial coupling is known, or to investigate the mechanism of heredity in forms in which the synaptic conditions are better understood.

The 'change of partners' observed in tetrad stages may have no significance for heredity in the sex in which linkage is complete, but may be associated with more important conditions, such, possibly, as breaks and re-fusions in the female of Drosophila, the male of Bombyx, both sexes of the rat, and in the development both of pollen nuclei and of ova in Primula and Lathyrus.

Culex may be expected to give no crossing over of factors in the male, if we may generalize so widely from conditions in Drosophila, but Culex is a fly of relatively lower order taxonomically and somewhat nearer to the common stem of Diptera and Lepidoptera. We may perhaps expect to find some connecting link between Drosophila, in which linkage is complete in the male only, and Bombyx in which linkage is complete in the female only.

The cytological results in Culex thus far throw no light upon these questions.

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EXPLANATION OF PLATES

The drawings were made with a camera lucida, using a compensating ocular 20X and a Zeiss homogeneous immersion apochromatic 2.0 mm. objective. They were drawn at a magnification of 3000 from the specimens and enlarged to 6000. The drawings have been reduced one-third in reproduction, giving a final magnification of 4000 diameters.

The drawings are listed below to show which are taken from the same individual, the sex and stage of development of the individual, and the fixation.

INDIVIDUAL NO.	SEX	STAGE OF DEVELOPMENT	FIXATIVE	DRAWINGS NUMBER
1	♂	Larva	Flemming	1, 7, 10, 11, 12, 13, 27
2	♂	Pupa	Flemming	2 29, 30, 31
3	♂	Pupa	Flemming	3, 4, 5, 6,
4	♂	Larva	Flemming	8, 9
5	♂	Pupa	Flemming	14
6	♂	Larva	Flemming	15
7	♂	Pupa	Flemming	16, 34, 35, 36, 39, 44
8	♂	Pupa	Flemming	20, 21, 22
9	♂	Pupa	Flemming	17, 18, 19, 23, 25
10	♂	Pupa	Flemming	24, 26, 32, 33, 90, 91, 92, 93
11	♂	Larva	Flemming	28
12	♂	Pupa	Flemming	37, 38, 42
13	♂	Pupa	Flemming	43, 45, 46, 47
14	♂	Pupa	Flemming	41, 71
15	♂	Pupa	Flemming	48, 49, 50, 51, 54, 76, 77, 78, 79, 80, 81, 86, 87, 88, 89, 95
16	♂	Pupa	Flemming	52, 61, 97
17	♂	Pupa	Flemming	53, 55, 56, 57, 58, 59, 60, 62, 64, 65, 94
18	♂	Pupa	Flemming	63
19	♂	Pupa	Flemming	66, 67
20	♂	Pupa	Flemming	68, 69, 70, 72, 73, 74, 75
21	♂	Pupa	Flemming	82
22	♂	Pupa	Picro-formol acetic	83, 84, 85
23	♀	Pupa	Flemming	100, 101
24	♀	Pupa	Flemming	103, 104
25	♀	Adult	Flemming	98, 99, 102, 105, 106, 111
26	♀	Adult	Picro-formol acetic	107, 108, 110
27	♂	Larva	Flemming	115, 116
28	?	Larva	Flemming	112
29	♂	Larva	Flemming	113, 114, 119
30	♂	Larva	Flemming	117, 118.

PLATE 1

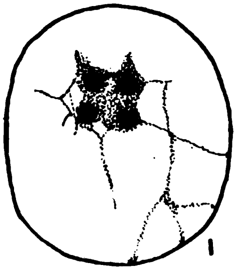
EXPLANATION OF FIGURES

1 to 10 Spermatogonial prophases, early and late. Figure 9 shows a possible sex element.

11 to 14 Spermatogonial metaphase. Stage A. Figures 12 and 14 show possible sex elements.

15 Transitional from Stage A to Stage B.

16 and 17 Spermatogonial metaphase. Stage B.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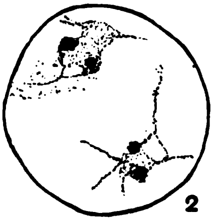
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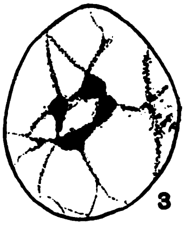
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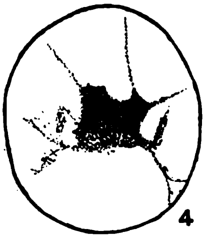
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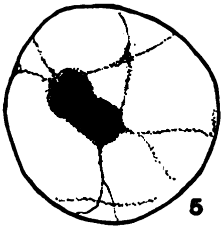
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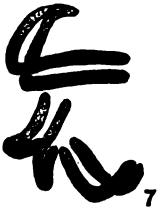
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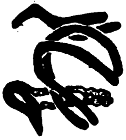
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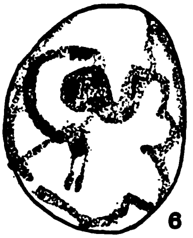
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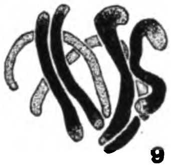
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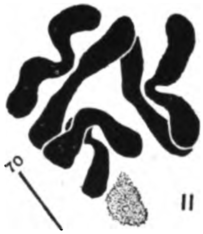

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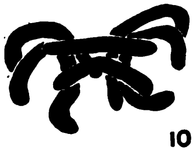
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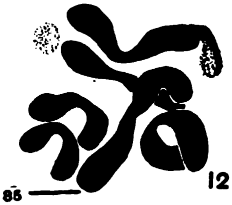

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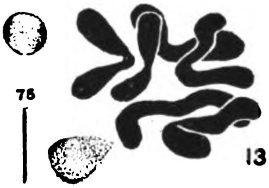

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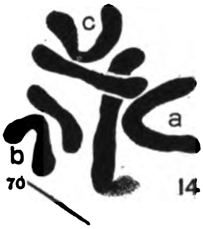

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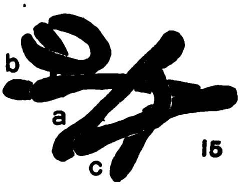

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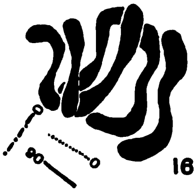

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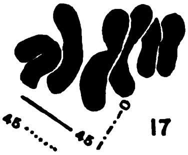

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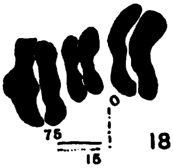
PLATE 2

EXPLANATION OF FIGURES

18 to 23 Spermatogonial metaphases.
24 to 27 Spermatogonial anaphases.
28 Spermatogonial telophase.
29 to 36 First spermatocyte growth stages.



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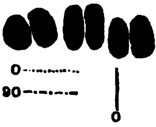

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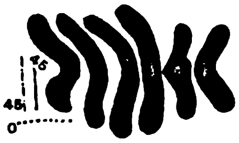

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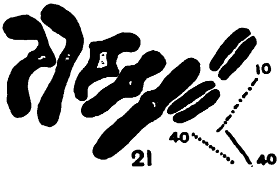

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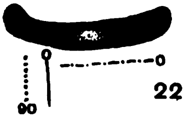

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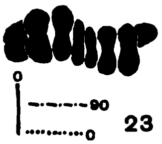

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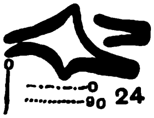

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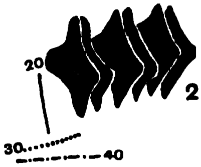

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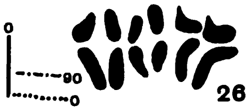

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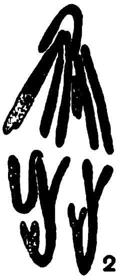

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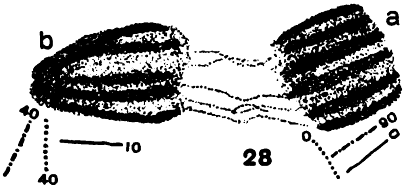

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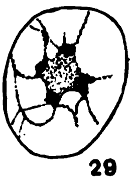

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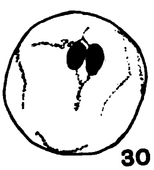

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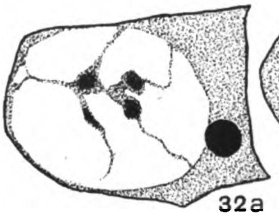

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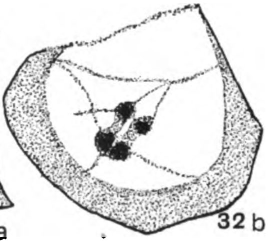

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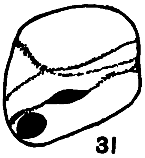

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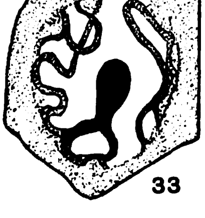

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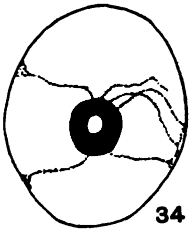

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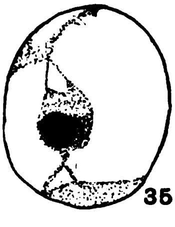

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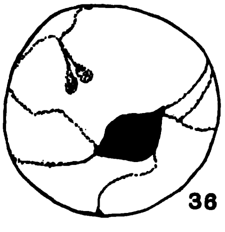

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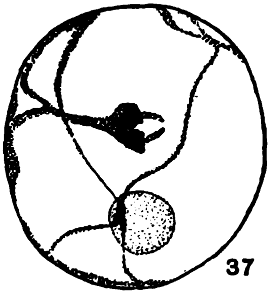
PLATE 3

EXPLANATION OF FIGURES

First spermatocyte prophases, showing nucleoli and terminal granules.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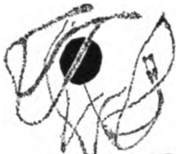
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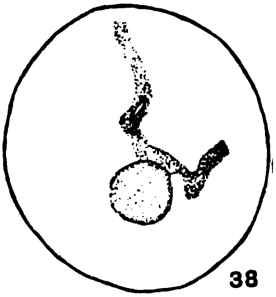
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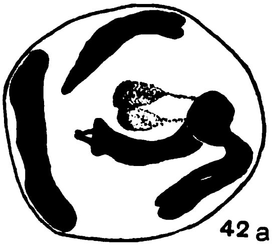
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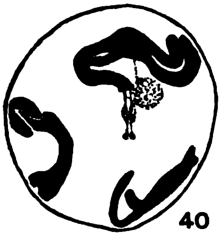
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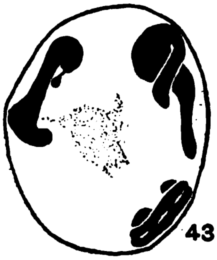
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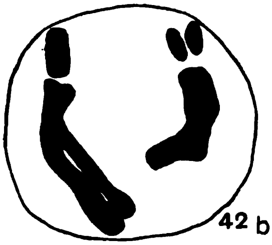
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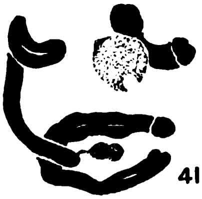
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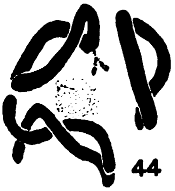
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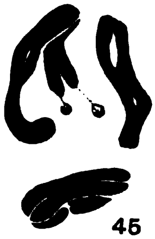
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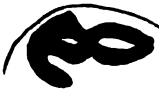
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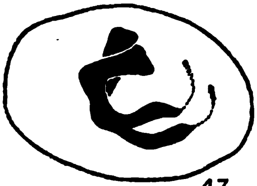
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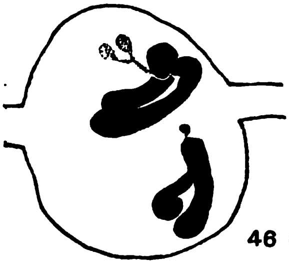
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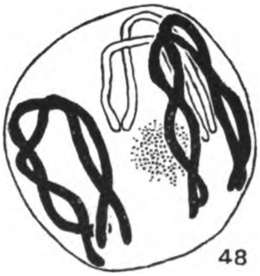
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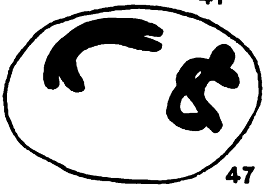
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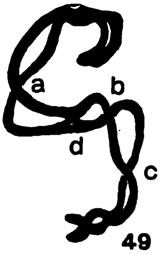
PLATE 4

EXPLANATION OF FIGURES

49 Prophase chromosome showing 'chiasma.'

50 to 64 Prophases, metaphases, and early anaphases of first spermatocytes. The tetrads act independently of each other in passing through these stages.



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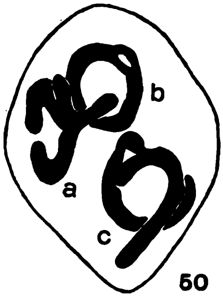

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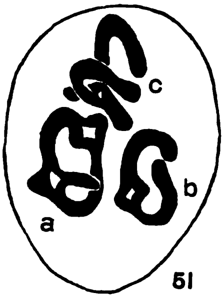

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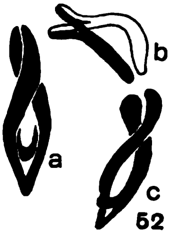

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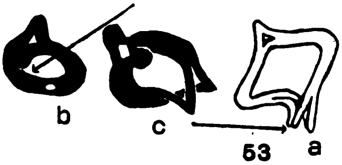

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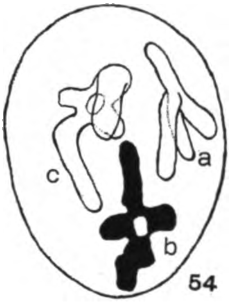

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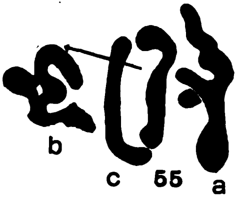

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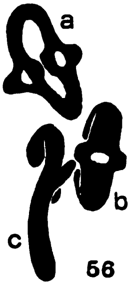

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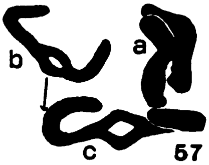

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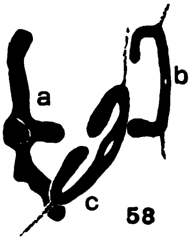

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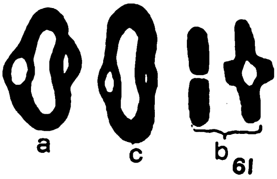

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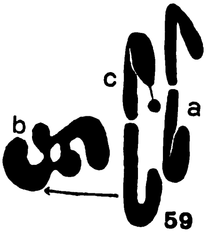

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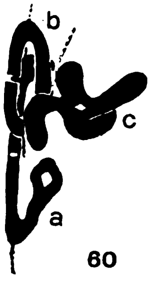

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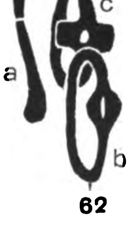

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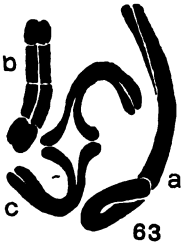

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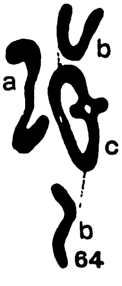

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PLATE 5

EXPLANATION OF FIGURES

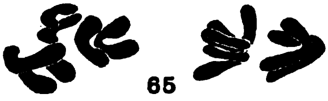
65 to 67 First spermatocyte late anaphases. Figure 65 is an oblique view. Figure 66 shows polar views of the two poles. Figure 67 is an optical section at one pole.

68 Very late anaphase of first spermatocyte. *a* and *c* show the chromosomes, while *b* is a cross section of the spindle fibers.

69 First spermatocyte telophases. *a* and *b* show the upper pole; *d* and *e*, the lower; *c* is a cross section of the spindle fibers.

70 to 75 First spermatocyte telophases.

76 to 79 Interkinesis nuclei showing nucleoli and chromosomes.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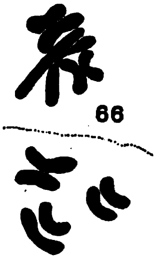
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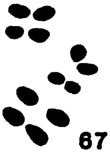
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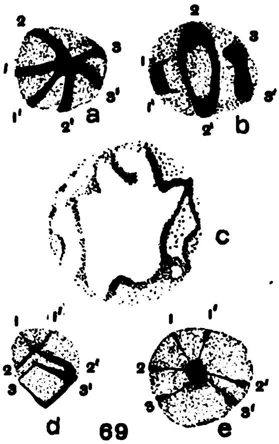

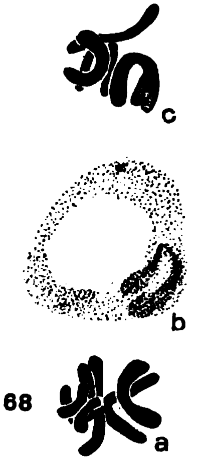

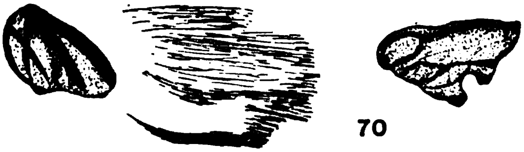
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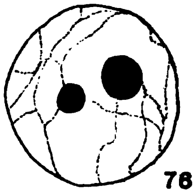
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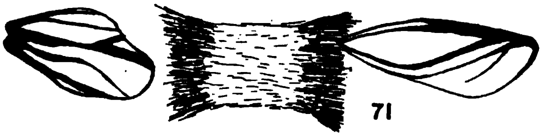
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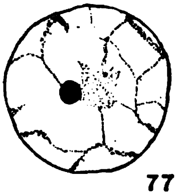
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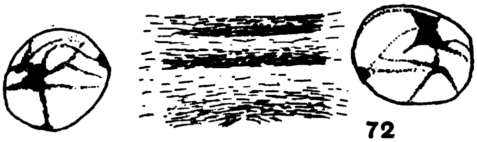
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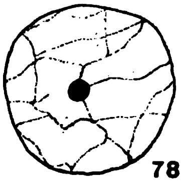
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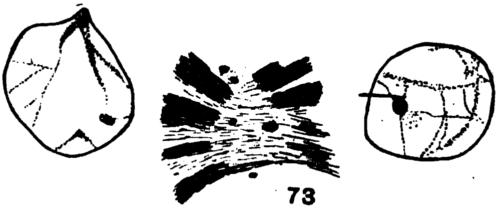
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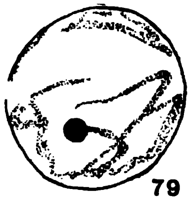
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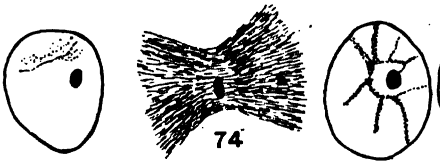
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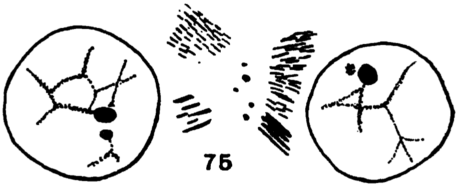
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PLATE 6

EXPLANATION OF FIGURES

80 Interkinesis nucleus showing smaller nucleolus dividing.

81 to 85 Prophases of second spermatocyte showing three pairs of chromosomes.

86 Metaphase of second spermatocyte.

87 and 88 Anaphases of second spermatocyte.

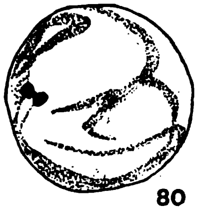
89 Telophase of second spermatocyte.

90 to 93 Spermatids.

94 and 95 First spermatocyte cells showing karyokinesis without cytokinesis.

96 Pycnotic cells.

97 Pathological overgrown cell among the first spermatocytes.



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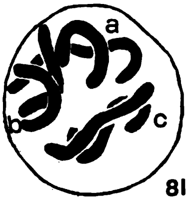

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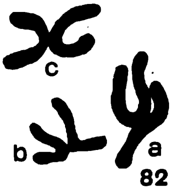

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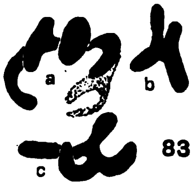

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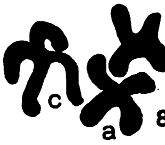

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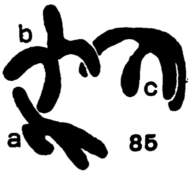

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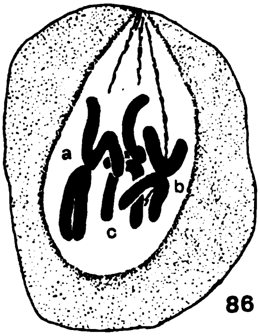

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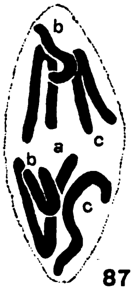

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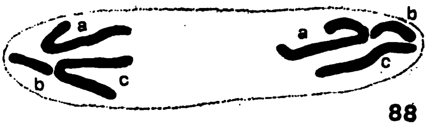

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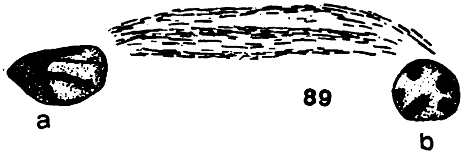

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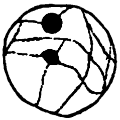

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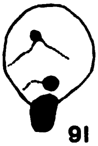

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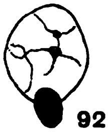

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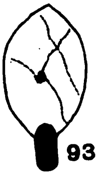

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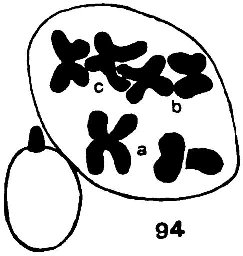

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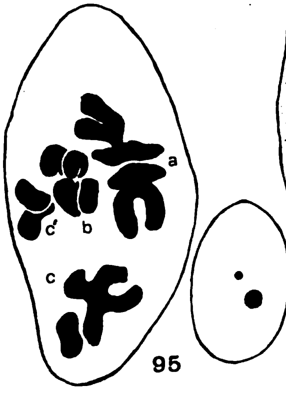

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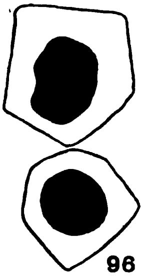

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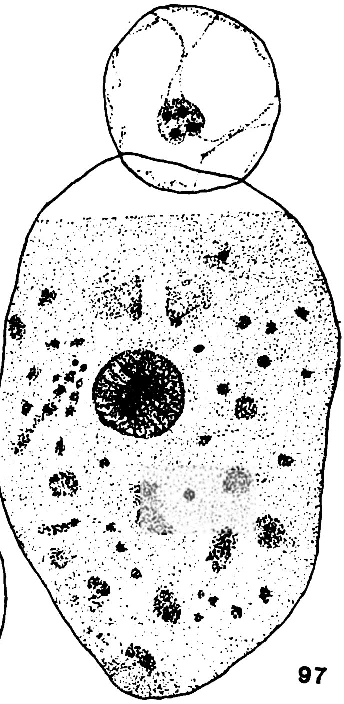

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PLATE 7

EXPLANATION OF FIGURES

98 and 99 Ovarian prophases showing three pairs of chromosomes. The members are more or less fused. One of the pairs in each cell shows terminal granules (?).

100 Small pair showing each member split in unusual ovarian prophase.

101 The three prophase pairs showing each member split, from same section as figure 100.

102 to 104 Ovarian metaphases. Stage A.

105 and 106 Ovarian metaphases. Stage B.

107 to 111 Ovarian anaphases.

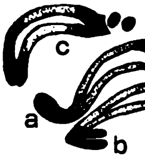
112 to 119 Somatic mitoses from thoracic hypodermal cells of larvae.

112 and 113 Prophases.

114 and 115 Metaphases. Stage A.

116 and 117 Metaphases. Stage B.

118 and 119 Anaphases.



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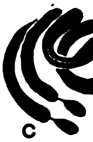

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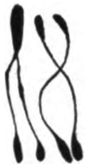

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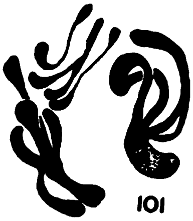

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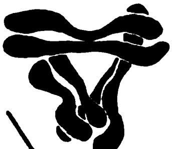

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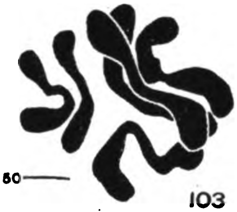

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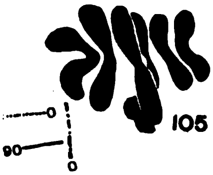

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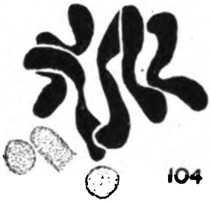

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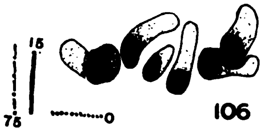

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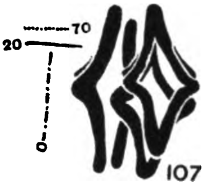

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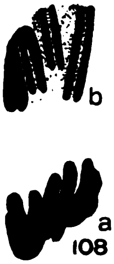

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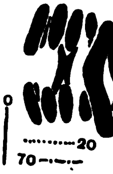

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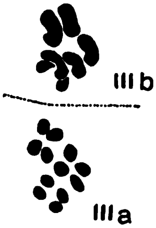

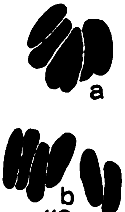

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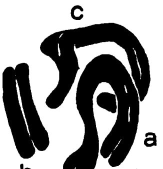

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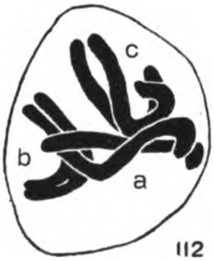

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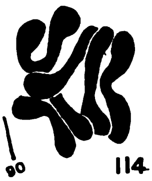

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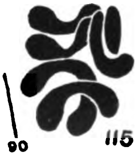

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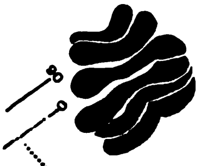

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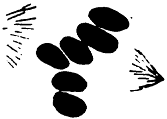

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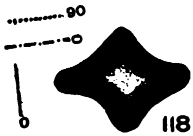

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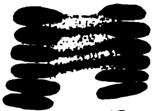

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